AFRL-RX-TY-TR-2008-4511

EFFECT OF ENVIRONMENTAL PARAMETERS ON THE BIOCIDAL PERFORMANCE OF IODINE-TREATED FILTERS

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JANUARY 2008

Final Report for 1 August 2006 – 31 July 2007

Air Force Research Laboratory
Materials and Manufacturing Directorate
Airbase Technologies Division
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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
16-JAN-2008	Final Technical Report	01-AUG-2006 – 31-JUL-2007

4. TITLE AND SUBTITLE

Evaluation of the Performance of Iodine-Treated Biocide Filters Challenged with Bacterial Spores and Viruses

5a. CONTRACT NUMBER

FA8561-05-C-0136

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

62384BP

6. AUTHOR(S)

Wu, Chang-Yu; Lee, Jin-Hwa; Riemenschneider, Lindsey; and Theodore, Alexandros D.

5d. PROJECT NUMBER

ARMT

5e. TASK NUMBER

00

5f. WORK UNIT NUMBER

ARMT0047

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Air Force Research Laboratory
Materials and Manufacturing Directorate
139 Barnes Drive, Suite 2
Tyndall Air Force Base, FL 32403-5323

10. SPONSOR/MONITOR'S ACRONYM(S)

AFRL/RXQL

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

AFRL-RX-TY-TR-2008-4511

12. DISTRIBUTION/AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A: Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

Technical POC: Joe Wander, AFRL/RXQL. Document contains color images.

14. ABSTRACT

Iodine-treated filter media were challenged at a face velocity of 14.2 cm/s by *Bacillus subtilis* spores and MS2 bacteriophage aerosols generated from a Collison nebulizer. The novel medium displayed excellent viable removal efficiency (VRE) for bacterial spores with a negligible pressure drop in various environmental conditions, suggesting an alternative to HEPA filters for the removal of bacterial spore aerosols.

Different filter media tested for viral aerosols showed VRE >90% but lower than that for bacterial spores, with negligible variation in the pressure drop. The iodine-treated filter showed higher VRE than that of the untreated filters, possibly due to sublimation and dissolution of iodine molecules at HT/LRH and RT/MRH, respectively. Insignificant difference was observed between the (minimal) survival fractions of viral particles caught on iodine-treated and untreated filters at the same environmental condition, (continued, p. ii)

15. SUBJECT TERMS

aerosol, bacillus subtilis, bacteriophage, filter, iodine, protection, spore, virus

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Joe Wander, RXQL
U	U	U	SAR	85	19b. TELEPHONE NUMBER *(Include area code)*

Reset

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

14. Abstract (Concluded):

indicating negligible effect of iodine treatment of these particles. Encasement of microorganisms is a possible problem in the efficacy of an antimicrobial filter because the viability of microorganisms is preserved when shielded from disinfection agents.

A condensation nuclei concept using water vapor was tested and shown to be an effective device for enhancing condensation and improving efficiency of collection of virus-containing aerosols smaller than ~100 nm. However, the problem of losses to reentrainment of bioparticles as air bubbles break in the impinger remains to be solved.

EXECUTIVE SUMMARY

A. OBJECTIVE:

Two objectives are considered: (1) to evaluate the performance of iodine-treated biocidal filter media for bacterial spores and viral aerosols as a possible component of gear protective against bioterrorism and pathogenic airborne biological agents, (2) to develop a condensation nuclei device for improved collection efficiency of viral aerosols.

B. BACKGROUD:

The increasing threat of biological warfare and the spread of airborne pathogens have attracted public attention to bioaerosols and created an opportunity for development of methods for respiratory protection. Filter media combining mechanical filtration and disinfection capacity of iodine allow protection against bioaerosols with high removal efficiency and lower pressure drop (ΔP) than conventional filter media.

No current sampling methods are adequate to collect ultrafine viral aerosols. A system that can cause ultrafine particles to grow by condensation will provide an increase in collection efficiency of ultrafine bioaerosols.

C. SCOPE:

The efficiency of iodine-treated biocidal filter media was evaluated under various environmental conditions to ensure reliability for practical application. Removal efficiency and the viability of collected microorganisms on the filter were investigated to determine the effectiveness of the iodine-treated media. A condensation nuclei device was designed and built. The performance of the condensation nuclei device was evaluated by growing NaCl and MS2 bacteriophage aerosols and collecting them in a downstream impinger. Reaerosolization of viral particles from the impinger was also explored to assess the net benefit to be realized by the new device.

D. METHODOLOGY:

The iodine-treated filter media were challenged at a face velocity of 14.2 cm/s with *Bacillus subtilis* spores or MS2 bacteriophage aerosols generated by a Collison nebulizer. The bacterial spore aerosols entering and penetrating the test filter were collected and classified by using an Andersen six-stage impactor. For viral aerosols, the impactor was replaced by the AGI-30 impinger. Temperature and relative humidity of the system were adjusted by using a heating jacket and dry or humid air, respectively. After the filtration experiment, the viability of microorganisms collected on the filter was investigated by

enumeration of extracted microorganisms from the filter using a vortexing method. The effect of free iodine on viability in the vortexing solution was factored in the correction of the vortexing experiment results.

To evaluate the condensation nuclei device, NaCl and MS2 bacteriophage aerosols were generated from the Collison nebulizer. The aerosols were dried in the diffusion dryer and then delivered into the condensation nuclei device. AGI-30 impingers were employed to collect the enlarged aerosols in the device, and the collection medium was analyzed by ion chromatography and by bioassay. For the reaerosolization experiment, clean air was drawn into the impinger containing a known concentration of virus suspension and the reaerosolized particles from the impinger were measured by a Scanning Mobility Particle Sizer (SMPS). This experiment was conducted by varying the flow rate and the virus concentration in the impinger.

E. TEST DESCRIPTION:

The iodine-treated and untreated filters were tested with bacterial spore and with viral aerosols. Three sets of environmental conditions were selected for testing: room temperature (23 ± 2 °C) and high relative humidity (95 ± 5 %) (RT/HRH); RT (23 ± 2 °C) and moderate RH (50 ± 5 %) (RT/MRH); and high temperature (40 ± 2 °C) and high RH (95 ± 5 %) (HT/HRH). The loss of virus infectivity due to sublimation and dissolution of iodine was investigated by conducting filtration experiments at HT/LRH and RT/MRH with impingers containing a known concentration of virus suspension. After 4 hrs or 10 hrs of filtration experiments, the filters were vortexed with sterile deionized water to extract microorganisms collected on the filter. The viability of microorganisms extracted from the filter was corrected for the effect of free iodine released from the iodine-treated filter during vortexing.

The efficiency of the condensation nuclei device was verified by comparing the sodium concentration in the impinger from NaCl aerosols captured with and without utilizing the device. It was also verified by comparing the MS2 concentration with and without the device. Reaerosolization from the impinger was investigated at varying flow rates, 2–10 Lpm, and virus concentrations, 10^2–10^8 PFU/mL.

F. RESULTS:

Both iodine-treated (JT-70-20XP-10T-100) and untreated (JT-70-20XP-100) filters exhibited high viable removal efficiency (VRE, > 99.99%) for bacterial spore aerosols in various environmental conditions. Pressure drag of the tested filter was much lower than

that of a glass fiber filter (6×10^3 Pa/(m/s) vs. 4×10^4 Pa/(m/s)). At RT/LRH, survival fractions of the iodine-treated and untreated filter were $6.9\times10^{-4}\pm1.6\times10^{-4}$ and $2.5\times10^{-3}\pm1.4\times10^{-3}$, respectively. However, at RT/HRH and HT/HRH, survival fractions on the treated media were statistically the same as the untreated control at RT/LRH.

For viral aerosol experiments, filter media (polyester–cotton coated with 125 g/m^2 tri-iodide resin) were supplied by AFRL. Filter quality of the test filters (16 kPa^{-1}) was greater than that of a glass fiber HEPA filter (5 kPa^{-1}). The physical removal efficiency (PRE) of the filters was 32 ± 3 % for aerosols ranging from 11.3 to 187.7 nm. VRE performance of iodine-treated (93.6 ± 1.2 %) and untreated (91.7 ± 0.9 %) filters was similar at RT/LRH. At HT/LRH and RT/MRH, the iodine-treated filter presented a higher value than that at RT/LRH; whereas untreated filters performed the same as at RT/LRH. Sublimation and dissolution of iodine molecules were available for release from the iodine-treated filter at HT and MRH, but the observation of attenuation of viability of MS2 only at warm or damp conditions will require additional observations before it can be reconciled with earlier reports. At the same environmental conditions, there was no significant difference between the survival fraction of MS2 collected on the iodine-treated and untreated filters. The insignificant effect of iodine on the infectivity of collected MS2 aerosols might be explained by the shielding effect of aggregated/encased MS2 particles collected on the filter or by exhaustion of surface I_3^- depots as above.

The condensational growth unit increased the fraction of particles collected in the impinger. The collection efficiency of NaCl was thus improved by 25% to 48%, whereas collection efficiency of MS2 increased over 80% compared to collection without the growth unit. During reaerosolization experiments, increasing airflow rate significantly increased the rate of reaerosolization of virus particles from the impinger. A surprising result was that reaerosolization of particles showed a decreasing trend at concentrations greater than 10^6 PFU/mL in the impinger.

F. CONCLUSIONS:

The novel iodine-treated filter has an excellent VRE for bacterial spores with a negligible ΔP in various environmental conditions. Behavior of the iodine-treated filter medium presents an alternative to the conventional HEPA filter for the removal of bacterial spore aerosols. Deactivation of the collected bacterial spores is only slightly enhanced by the presence of the iodinated resin. The use of the iodine-treated filter may provide an economical way to remove bioaerosols and a solution to the problem of air filters' being a potential source of airborne microbial contamination.

Different filter media challenged with viral aerosols showed VRE higher than 90% but lower than that for bacterial spores, with negligible change in ΔP. The iodine-treated filter showed higher VRE than that of the untreated filters; however, with the medium samples and conditions we used, the increased activity appears to be due to iodine sublimation and dissolution of iodine molecules at HT/LRH and RT/MRH, respectively, rather than near-contact transfer. Insignificant difference was observed between the minimal survival fractions of viruses on iodine-treated and untreated filters, respectively, at the same environmental condition. We propose to repeat these measurements with virgin samples of the media. We tentatively interpret this to mean that iodine is no more effective than oxygen in penetrating larger aggregates at whose center a few virons are able to survive, but cannot exclude the possibility that earlier usage exhausted surface concentrations of available iodine. For treatment of viral aerosols, the efficiency of the iodine-treated filter can apparently be improved by manipulating conditions (*i.e.*, raising temperature and humidity) entering the filter to promote release of iodine from deeper reservoirs.

Initial testing of the condensation nuclei device clearly demonstrated feasibility of driving condensation to enhance fine-particle [virus] collection.

G. RECOMMENDATIONS:

Further study is needed to determine the effectiveness of iodine-treated filter media in the real world and specifically to clarify the observations reported for viral VRE. The interference of dust loading should be examined to characterize the extent to which the presence of materials that can interact with the active sites of the filter medium affects PRE and VRE. Initial experiments have shown that post reactions with I_2 can be eliminated by adding sodium thiosulfate to the collection medium. The use of thiosulfate solution as a collection medium is recommended to quench the reaction of iodine for future experiments that do not back the iodinated-resin filter with an adsorber.

Using particles with a better-defined size range is proposed to quantify performance of the condensation nuclei device. The device can be tested with MS2 bacteriophage and non-biological particles of similar size to examine the effect of conditions on viability of bioaerosols. Reaerosolization should be examined using higher viral concentrations in the impinger.

PREFACE

This report was prepared by the Aerosol and Particulate Research Laboratory, Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611-6450, under Contract Number FA8650-06-C-5913 for the Air Force Research Laboratory (AFRL/RXQ), 139 Barnes Drive, Tyndall AFB, FL 32403-5323.

This is an annual report is submitted to AFRL/RXQ. This report describes the work conducted from August 2006 to July 2007. The Air Force Technical Program monitor was Dr. Joseph D. Wander. We greatly appreciate the assistance of Triosyn Corp., Dr. Dale Lundgren and Dr. Samuel Farrah at the University of Florida, and Dr. Jean Andino at the Arizona State University, for their advice. We also thank Katherine M. Wysocki and Christiana Nicole Lee from the Department of Chemical Engineering at the University of Florida for their assistance in the laboratory.

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1 INTRODUCTION

1.1 Objective

There were two objectives in this first year of study. First, to determine the ability of the iodine-treated biocidal filter to capture and to devitalize bacterial spore and viral aerosols under various environmental conditions—the ultimate goal is to evaluate the possible enhancement of respiratory protection that the iodine-treated filter can provide against bioterrorism and other sources of airborne pathogens. Second, to explore the application of condensational growth as a tool to enhance the collection of ultrafine bioaerosol particles—to go to practice it will be necessary also to assess and find ways to mitigate the extent of reaerosolization of virus particles following collection in the impinger.

1.2 Background

1.2.1 Biological threat

Increasing concerns about bioterrorism after the 18 September 2001 anthrax attack and episodes of spreading airborne pathogens—*e.g.*, Severe Acute Respiratory Syndrome (SARS), H5N1 and Avian/Bird Flu viruses—have drawn attention to bioaerosols and protection methods. Because the production of bacteria having massive toxins and virulent strains of virus is easy and inexpensive, practical quantities of biological warfare agents (BWAs) can be made by small groups and terrorist organizations. The spread of BWAs is silent—they are invisible, odorless, tasteless, and generally will remain undetected until symptoms develop in infected people. Chemical agents spread in a downwind area near the point of release; in contrast BWAs can spread widely throughout a city or region [1].

The spread of airborne pathogens is an environmental situation driving public awareness of bioaerosols. For instance, SARS—a viral respiratory illness—is caused by a corona virus for which there is currently no vaccine and no cure. During the first few months following the first report of SARS in Asia in February 2003, the virus spread to more than two dozen countries in North and South America, Europe and Asia. Transmission of the SARS virus is suspected to occur as sneezing or coughing of an infected person creates droplets containing the virus, which subsequently deposit on mucous membranes of the mouth, nose, or eyes of nearby persons [2]. Besides SARS, infections transmitted by the respiratory route include tuberculosis, mumps, measles,

pneumonia and influenza, in addition to many veterinary and agricultural diseases [3].

1.2.2 Bioaerosols

Bioaerosols are aerosols of biological origin including viable bacteria, viruses, fungi and algae, as well as such nonviable materials as pollen, endotoxins, mycotoxins and various allergens [4]. They are associated with a wide range of adverse health effects such as allergies, organic toxic syndrome, asthma and other respiratory illnesses. Bioaerosols must be viable to be infectious, but viability is not a prerequisite to allergenic and toxic effects [5]. Non-viable bioaerosols cause such allergic reactions as hay fever, rhinitis, and asthma by contact and inhalation [6]. Humans sneezing and coughing comprise one of the most important sources of bioaerosols. Thousands of droplets 1~10 μm in diameter containing viable microorganisms released by a person will quickly contract by evaporation to form droplet nuclei, which remain suspended in air for long periods of time and travel considerable distances as or by attaching to aerosols. In particular, respiratory viruses appear to be spread mainly by droplet nuclei [7, 8]. Due to droplet encasement, virus infectivity can be shielded from external forces (*e.g.*, drying, sunlight, and temperature) [9]. In indoor environments, microorganisms are also free from factors inducing the destruction of microbes, which prolongs the survival of airborne microbes. Ultraviolet radiation in direct sunlight kills microorganisms. Oxygen and air pollutants may also act to destroy microbes.

Among the various species of microbes, bacterial spores and viruses are of special concern because of their unique properties. In adverse environmental conditions, certain species of bacteria can survive by forming endospores, which exhibit incredible longevity and resistance to environmental stress [10]. Germination and the outgrowth of vegetative cells are initiated when the endospores encounter an appropriate environmental trigger, *e.g.*, a simple amino acid or riboside [11]. Bacterial spores are highly resistant to deactivation, as by heat, radiation and chemical agents. Specific properties of spores contributing to their resistance include low water content in the core and saturation of the spore DNA with a group of small, acid-soluble spore proteins (SASP) of the α/β-type [12, 13]. Thus, spores have been classified as a group of bioagents for which treatment and disinfection are specially challenging. The singularity of viruses is their size (viz., 20–300 nm as a single naked virus). In the natural environment they typically occur in a wide range of particle sizes due to aggregation of several single viruses or attachment to various non-biological particles (*e.g.*, dust) in the air. The resultant effect is that viruses are typically present in the submicron size range [14]. Particles this size exhibit high penetration in a filtration system

because they follow the airflow streamlines—the condition for minimum efficiency of capture because it falls in the gap between regimes of efficient mechanisms of collection by diffusion and by interception or impaction. Finally, by shielding internal virus particles, encasement of viruses in other constituents of the particle can enhance survival in both Nature and the presence of antimicrobial agents.

1.2.3 Filtration

Filtration is the commonest method to remove aerosols because it combines the advantages of simplicity, versatility and low cost. Air filtration has been used extensively in various applications, including clean rooms and respiratory protection [15]. Two key factors determine the effectiveness of a filter: (a) collection efficiency (CE), the fraction of particles retained in the filter, and (b) pressure drop (ΔP), which is related to energy cost. A high-efficiency particulate air (HEPA) filter has high CE of aerosols, greater than 99.97% for the nominal most-penetrating particle size (MPPS), 0.3 μm (300 nm). However, high ΔP and growth of collected microorganisms are concerns in its bioaerosol applications. Under suitable growth conditions—sufficient nutrients, proper humidity and temperature—collected microorganisms can proliferate, causing illness and allergies by re-entrainment into the air [6]. The HEPA filter also poses a hazard to workers who handle microorganism-loaded filters for disposal. Even though HVAC (Heating, Ventilating and Air Conditioning) systems prevent the contamination of indoor air by environmental bacteria and spores entering from outdoors, once their growth occurs in the system, they can appear in returned air at a higher level than in the outdoor air [16]. It has been shown that at sufficient relative humidity fibrous building materials including insulation substances and ceiling tiles provide nutrients for the growth of microorganisms [17, 18]. Research into the effect of air filter media on the viability of bacteria showed that fiber materials did not have an inhibitory effect on the survival of microorganisms even if they do not grow [19]. Sensitive cells lose their viability in less than three days after collection, but resistant bacteria such as B. subtilis spores can retain viability on the filter for a much longer time [20]. As previously mentioned, the complex structure of bacterial spores protects cellular components by developing antimicrobial resistance. However, low concentrations of chemical germinants can cause the spores to germinate making them vulnerable to antimicrobial treatment [11].

Deactivation of collected microorganisms is important for two reasons: first to

prevent contamination of ambient air by re-entrained microbes; second is to extend the lifetime of the filtration system by preventing proliferation of microorganisms in the filter. Therefore, in recent years there have been efforts to incorporate antimicrobial materials into air filters to destroy or inhibit the growth of microorganisms [21, 22].

1.2.4 Iodinated resin filter media

Iodine is one of the halogens and, like chlorine, it exerts biocidal effect as a strong oxidant. It has been used in water treatment, where it has some advantages over chlorine: greater chemical stability of the agent, less reactivity with organic nitrogenous contaminants found in water. Iodine is used by the military, in developing countries, and in emergency or temporary situations for portable water purification. Iodine is available in various forms, including solution, tablets and iodine resins [23]. Iodinated resins were developed to deliver release-on-demand disinfection. Iodine can be attached to a quaternary ammonium strong base cationic resin in the form of triiodide (I_3^-) and pentaiodide (I_5^-) anions [24]. These iodinated resins contain polarizable iodine complexes and microorganisms are negatively charged, leading to postulation of a mechanism of attraction and transfer of I_2 molecules during near-contact encounters [25]. After enough encounters, microorganisms are proposed to lose viability due to protein denaturation. Studies on the disinfection capacity of iodine resin filter for treatment of bacteria and viruses in water were conducted three decades ago and disinfection capacities over 99.99% were reported [24, 26–28]. However, only limited studies have been reported on the disinfection capacity of iodinated-resin filters for air treatment [29, 30]. Iodine-treated filter media combine mechanical filtration and disinfection by I_2 to treat microbial contamination of air, with the goal of reducing health risk by both capturing microorganisms and devitalizing most of those that penetrate. Iodine released on demand deactivates microorganisms by oxidizing cell components and iodinating cell proteins [31].

1.2.5 Condensation nuclei device

Current bioaerosol sampling methods are unable to effectively sample airborne viruses because the typical particle size is 20–300 nm. Sampling efficiency of various sampling methods is less than 10% for the most challenging sizes of 10–100 nanometers [14]. If sampling methodologies do not provide accurate results, the discrepancy between measured and actual virus concentrations can potentially lead to disastrous decision errors

because the infectivity of viruses is measured as a minimum threshold.

The use of condensational growth to enhance collection efficiency is an established research method that has been applied to many inert ultrafine particles [32–34]. The condensation nuclei counter is a well known application of condensational growth to improve sampling [35]. Although condensational growth has been previously utilized to improve collection of inert particles in the nanosize range, the approach has not yet been proposed for airborne viruses. Thus the use of condensational growth for bioaerosol sampling is a new application of a proven technology. This novel bioaerosol collection method has been developed and disclosed through the University of Florida Office of Technology Licensing (oral disclosure UF#12430).

The main function of the condensational growth device (CGD) is to increase the size of ultrafine (<500 nm) bioaerosols, giving the particles sufficient size and mass to be collected efficiently using standard bioaerosol collection techniques [35, 36]. The CGD employs the use of condensation onto the ultrafine particle to the extent that the particle acquires a larger effective diameter (>500 nm) that is sufficient for high sampling efficiency by impaction. This is accomplished by using a supersaturated vapor–air mixture condensing onto condensation nuclei, which grow until a vapor–liquid equilibrium is reached [36–38]. Condensation nuclei can be either solid or liquid, and act in the CGD as a host for the saturated vapor. In the case of ultrafine bioaerosols, individuals or clusters of airborne viruses are capable of acting as condensation nuclei in the presence of supersaturated water vapor, initiating water condensation onto the viral particle's surface. Provided sufficient time, the virus or other bioaerosol is capable of growth to micron-sized droplets [34] and subsequent collection using standard sampling techniques.

Application of condensational growth methods should increase collection efficiency significantly, as seen in other research evaluating its use on inert particles [32–34]. However, there is another factor affecting the sampling of nanosized airborne viruses besides small particle size. The rupture of bubbles created in impingers is known to be a significant source of reaerosolization of particles from the collection medium, which will decrease net collection efficiency [39, 40]. A study of reaerosolization is needed to analyze the extent to which this mode of loss competes with the enhancement of physical collection efficiency afforded by the CGD.

1.3 Scope

In a previous AF report [41], activity of iodinated filter media against bacterial spore and viral aerosols was tested at room temperature (23 ± 2 °C) and low RH (35 ± 5 %). For real applications, evaluating the effectiveness of the iodinated filter media in various environmental conditions will clarify the benefit of such media as a component of protective gear against biological agents and airborne pathogens.

A prototype condensation nuclei device was designed, built and tested. Performance of this prototype CGD was evaluated by growing an NaCl aerosol before passing it through an impinger for collection. Reaerosolization of viral particles from the impinger was also investigated to assess the impact of this mode of loss on the capacity of the new device.

This report describes the performance of the iodinated biocidal filter for bioaerosols in various environmental conditions and the CGD for improving airborne virus collection.

2 APPROACH

Two sets of experiments were carried out in this study: (1) Performance of the iodinated biocidal filter was evaluated for bacterial spores and viruses in various environmental conditions. To accomplish this task, a filtration system was used to measure removal efficiency of the filter media and vortexing recovery experiments were conducted to measure the viability of microorganisms collected on the filter. (2) A prototype condensation growth device (CGD) was designed and built to deposit water vapor onto condensation nuclei to increase the size and thus improve the collection efficiency of viral aerosols. Initial tests of the CGD's performance were conducted.

Specifically,

The removal efficiency of the iodine-treated biocidal filter for bacterial spore and viral aerosols in various environmental conditions was evaluated.

The viability of bacterial spores and infectivity of virus particles collected on the iodinated biocidal filter medium was investigated.

The condensation growth device was demonstrated to improve collection efficiency by challenging it with NaCl and MS2 bacteriophage aerosols.

The extent of reaerosolization of virus particles following collection in the impinger was measured at several conditions.

3 EXPERIMENTAL

3.1 Test Microorganisms

Bacillus subtilis spores supplied by the Department of Microbiology and Cell Sciences at the University of Florida and MS2 bacteriophage (*Escherichia coli* bacteriophage ATCC® 15597-B1™) were used as challenge organisms. *B. subtilis* is a Gram-positive, non-pathogenic, rod-shaped bacterium 2.0–3.0 μm long and 0.7–0.8 μm wide [31]. *B. subtilis* spores are commonly used as a surrogate for *B. anthracis* spores, which were the bioterrorism agent used in 2001. The spore production and purification procedures are described in Appendix A.

MS2 is an un-enveloped, single-strand RNA, in the shape of an icosahedron with 27.5-nm single-size diameter, that infects male *E. coli* [26, 31]. Because they share similar physical characteristics, MS2 has been used as a surrogate for human pathogenic viruses [42]. However, in the selection of a model virus, its resistance to antimicrobial agents also should be considered because resistance to inactivation varies from one virus to another. Berg et al. [43], who studied the effects of the virucidal properties of iodine molecules on enteroviruses, reported that coxsackievirus strains and poliovirus type 1 are more resistant to iodine inactivation than is echovirus type 7. Another study reported that poliovirus type 1 and echovirus type 7 are more resistant to iodine inactivation than is hepatitis A [44]. Since both enterovirus and MS2 have no lipid components, MS2 is considered to have resistance to halogenation similar to that of such enteroviruses as poliovirus, coxsackievirus and echovirus.

3.2 Bacterial Spore Aerosols

Iodinated (JT-70-20XP-10T-100) and untreated (JT-70-20XP-100) filter media tested in this study as discs 47 mm in diameter were provided by AFRL. Triiodide, prepared by mixing stoichiometric amounts of I_2 and potassium iodide in a minimum amount of water, was contacted with a quaternary ammonium anion exchange resin to substitute the anion with triiodide. The preparation procedures are detailed by Messier [29].

3.2.1 Aerosol generation and environmental conditions

The experimental system for evaluating removal efficiency is shown in Figure 3.1. A six-jet Collison nebulizer (Model # CN25, BGI Inc.) was used to aerosolize the spore suspension with a flow rate of 7 Lpm. The spore suspension in the nebulizer was made by dispersing 0.1 mL of purified spore suspension in 150 mL sterile deionized water. The

aerosolized suspension was dried with filtered compressed air in a 2.3-L glass dilution chamber. A flow rate of 15 Lpm—which corresponds to a face velocity of 14.2 cm/s—was used for both the control and experimental streams and controlled by a calibrated rotameter. This face velocity corresponds to the nominal velocity used by the National I statute for Occupational Safety and Health (NIOSH) for testing 100-mm filters commonly used in respirators. Pressure drop across the test filter disc was monitored using a Magnehelic gauge measuring 0–10 in. H_2O and recorded every 20 minutes. An Andersen six-stage viable impactor (Model #10-820, Thermo Electron Corp.) was used to classify generated bacterial spores and those that penetrated the test filter. After sampling, glass Petri dishes filled with either nutrient or tryptic soy agar were removed from the impactor, reversed, and incubated for 24–36 hrs before enumeration of microorganism growth.

To prevent contamination of ambient air a glass fiber HEPA filter (Catalog # AP1504700, 47 mm, Millipore Co.) was placed downstream of the impactor to capture spores, if any, not collected by the sampler. Because the cut size of the sixth stage of the impactor (0.65 μm) is smaller than the nominal size of a *B. subtilis* spore (1 μm), it is unlikely that any spores remained in the air downstream of the impactor. However, spore fragments that were not removed by the impactor were removed by the downstream filter. Experiments were conducted at two sets of environmental conditions: room temperature (23 ± 2 °C) and high RH (95 ± 5 %) (RT/HRH), and high temperature (40 ± 2 °C) and high RH (HT/HRH). Increased disinfection efficacy of iodine was predicted at HT and at HRH due in part to enhanced evaporation and dissolution of iodine, respectively. For the HT experiments the dilution dryer was wrapped in an electronically controlled heating jacket. HRH was achieved by introducing humid dilution air into the system.

3.2.2 Viable removal efficiency

The viable removal efficiency (VRE) of the test filter was calculated by enumerating bacterial growth in agar plates of two impactors, one downstream of the test filter and the other for control, which has no test filter. The VRE was determined as:

$$\text{VRE}\ (\%) = \left(1 - \frac{N_{p,\upsilon}}{N_{E,\upsilon}}\right) \times 100 \qquad (1)$$

where $N_{E,u}$ is the total number of entering viable spores collected in the control and $N_{p,u}$ is the number of viable spores collected downstream of the test filter. To prevent overloading

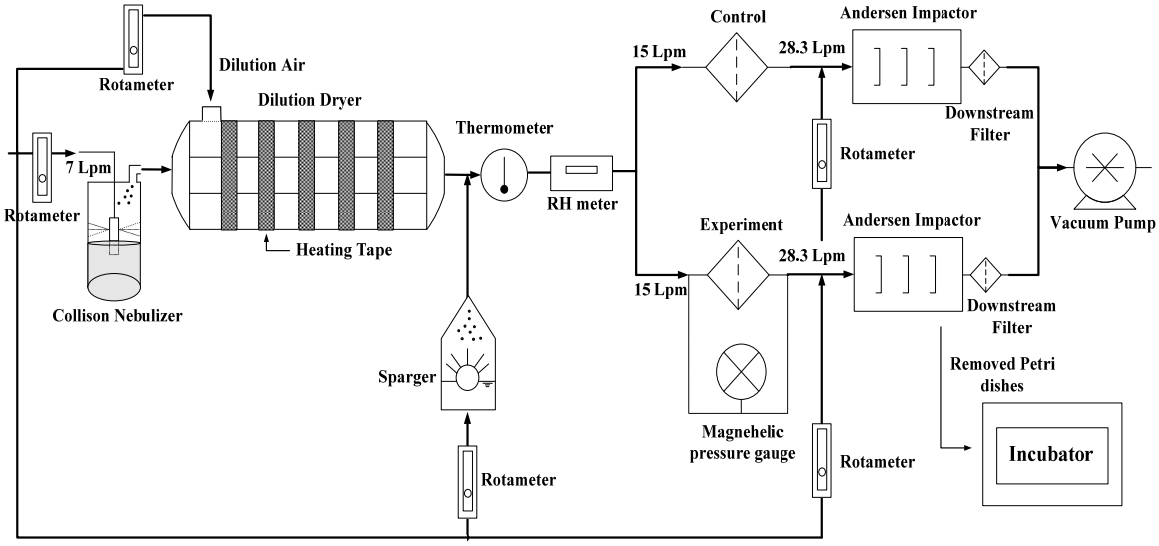


Figure 3.1. Experiment set-up for bacterial spore aerosol

of spores on the agar the entering bioaerosol concentration was measured by collecting spores at all six stages of the impactor with no test filter for the first and last 5 mins of an experimental run. The average number of colony-forming units (CFUs) measured for the two collections was used to determine the entering bioaerosol concentration for 2 hrs of experimental run. Due to the expected low penetration of spores through the test filter, the impactor downstream of the test filter contained only the sixth-stage agar plate. The agar plate was replaced with a fresh one every 20 mins for 2 hrs to avoid overloading and dehydration of the agar. At RT/LRH, five 2-hr trials were conducted; therefore, the total evaluation time for each filter was 10 hrs and three filters were tested (*i.e.*, 15 trials were recorded). However, due to the stability of results seen at RT/LRH and time constraints, only two iodine-treated filters were tested for two 2-hr runs in other environmental conditions (*i.e.*, four trials in all). Agar plates containing over 300 colonies were counted following the positive hole method recommended by the manufacturer. [45].

3.2.3 Viability of spores on the filter

After the filtration experiment, the test filter disc was removed from the filter holder in the experimental apparatus and subjected to the vortexing experiment to determine the viability of the spores collected on the filter. The filter was immersed in 40 mL sterile deionized water and agitated with a vortex mixer (Model # M16715, Barnstead).

After 1 min of vortexing, 1 mL of sample was withdrawn for measurement of spore viability and another 1 mL aliquot was withdrawn and measured after serial dilution. The same procedure was repeated after 2, 3, 5, and 10 mins of vortexing time without changing the solution. From this, the count of extracted spores, C_E, was determined as:

$$C_E = \frac{cfu}{10^{-n}} \times \frac{V_1}{V_2} \tag{2}$$

where *cfu* is the number of colony-forming units, V_1 is the volume of extraction fluid (1 mL), V_2 is the volume of diluted suspension spread on agar plate (1 mL), and *n* is the dilution factor. The total viability of the extracted spores was calculated by averaging the number of viable spores at all vortexing times. To compare the results of the iodinated and untreated filters, we defined survival fraction as the ratio of the number of spores extracted in the vortexing solution to the number of spores collected on the test filter (C_E/C_C).

In water, the resin surfaces may release iodine molecules, which can deactivate spores. Reaction of free residual iodine with spores in the vortexing solution would cause the measurement of deactivation on the filter to be exaggerated. This possibility was investigated by vortexing a clean iodine-treated filter for each designated time in water and inoculating the solution so produced with a spore suspension of known concentration. After 10 mins of exposure time, the viable spore concentration was measured to determine the free residual iodine effects. The concentration of iodine in the vortexing solution was also examined by the DPD (*N*, *N*-diethyl-*p*-phenylenediamine) colorimetric method adapted from *Standard Methods for the Examination of Water and Wastewater 4500-CI G* [46]. Ten mL of solution vortexed with the iodinated filter was analyzed at 530 nm using a DR/4000 V Spectrophotometer (Hach). Iodine in the solution reacts with DPD forming a pink color whose intensity is proportional to the total iodine concentration [47]. The effect of vortexing on the viability of spores was also investigated by performing the same vortexing procedure for differing times with a spore suspension of known concentration.

3.3 Viral Aerosols

New filter media different from those used in the bacterial spore experiments were supplied as sheets by AFRL. Iodinated (polyester–cotton coated with 125 g/m^2 of the triiodide resin) and untreated filters of the same thickness were tested as discs 47 mm in diameter.

3.3.1 Aerosol generation and environmental conditions

As shown in Figure 3.2, the filters were tested in the same experimental set-up used during the bacterial spore experiments to measure the VRE except that the sampling device was an AGI-30 impinger. Freeze-dried MS2 bacteriophage was suspended in filtered deionized water to a concentration of 10^8–10^9 PFU/mL to prepare the virus stock suspension. The virus suspension in the Collison nebulizer was prepared by adding 0.1 or 0.2 mL of virus stock suspension to 50 mL of sterile deionized water to produce a concentration of 10^5–10^6 PFU/mL. Test filters were challenged by viral aerosols in the same way as during the bacterial spore experiments. ΔP across each filter was recorded every 30 minutes. Viral aerosols entering and penetrating the test filters were collected in an AGI-30 impinger containing 20 mL of sterile aqueous solution. The collection medium in the impinger was replaced by fresh solution every 30 mins and assayed to investigate the virus concentration by serial dilution to an adequate count (*i.e.*, 30–300 PFU). Procedures for preparing the plaque assay medium are given in Appendix B. The downstream filter collected viral particles penetrating and reaerosolized from the impinger. Low (35 ± 5 %) and medium (50 ± 5 %) RH were used because maximum inactivation of MS2 aerosolized from 0.1*M* NaCl was reported to occur at 75 % RH [47]. As noted above, disinfection efficacy of iodine was expected to increase with increasing temperature, so we conducted experiments at two sets of environmental conditions: room temperature (23 ± 2 °C) and medium RH (50 ± 5 %) (RT/MRH), and high temperature (40 ± 2 °C) and low RH (35 ± 5 %) (HT/LRH).

The physical removal efficiency (PRE) was measured by using a Scanning Mobility Particle Sizer (SMPS, Model 3936, Shoreview, Minn., USA) in AFRL's laboratory. The particle size distribution (PSD) of the aerosols entering and penetrating the test filters was measured for 12 minutes through six consecutive 2-min recordings.

3.3.2 Removal efficiency

Removal efficiency of the test filters can be presented by PRE and VRE. The PRE (η_p) measured by using the SMPS was determined as:

$$\text{PRE (\%)} = \left(1 - \frac{N_p}{N_E}\right) \times 100 \qquad (3)$$

where N_E is the number of particles entering the filter and N_P is the number of particles penetrating the filter.

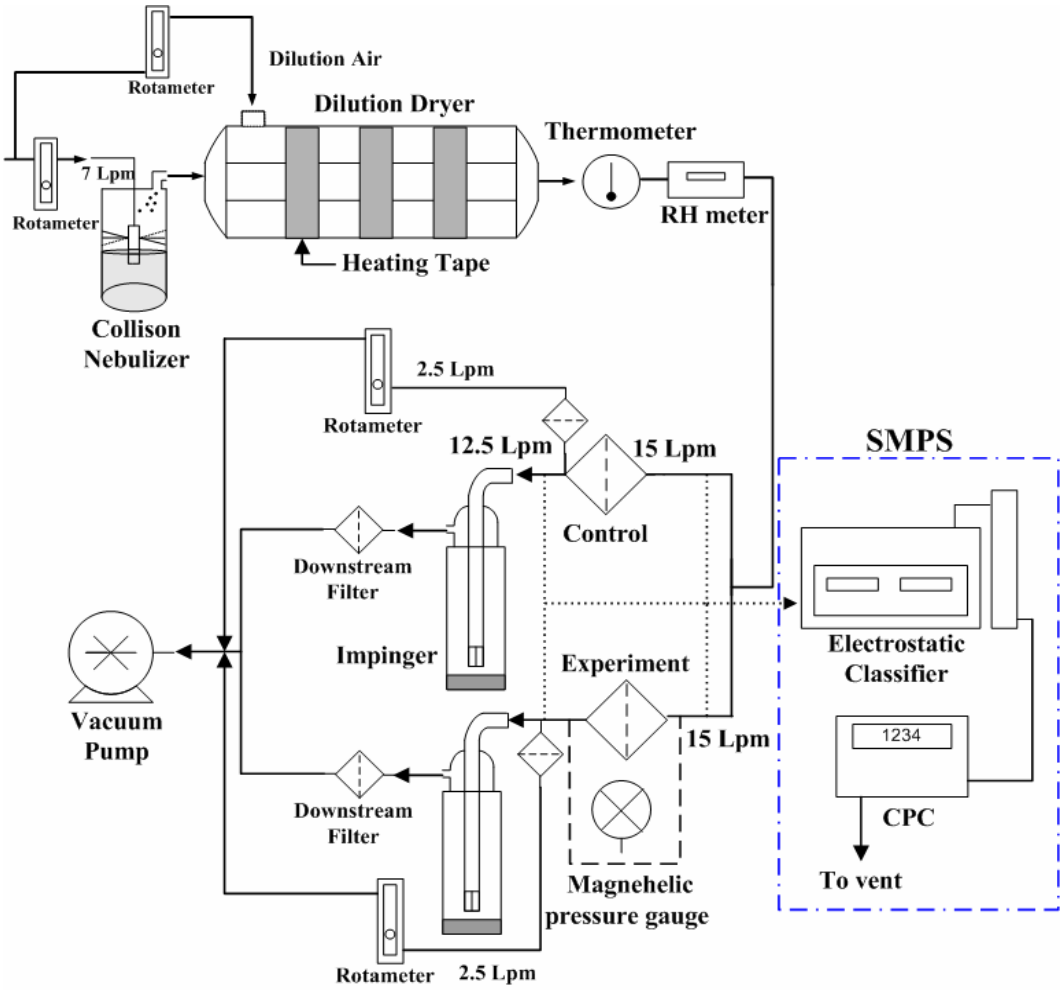


Figure 3.2. Experimental set-up for the (a) viable removal efficiency (b) physical removal efficiency of the test filters

The VRE describes the loss of infectivity of viruses collected in the impingers. The VRE (η_v) was determined by counting plaques on each Petri dish of both control and experimental impingers, and calculated as $100(1\text{-}N_P/N_E)$ % as in Eq. (3). In calculating virus concentration, the dilution factor was used to correct for the number of dilutions of the impinger solution to achieve a countable population. Thus, the viral concentration, C_v (pfu/mL), in the impinger was determined as:

$$C_v = \frac{pfu}{10^{-n} \times V} \tag{4}$$

where *pfu* is the number of plaque-forming units, *V* is the volume of diluted solution, and *n* is the dilution factor. The final mean viral concentration was determined by averaging all values in each dilution. Since the results seen at RT/LRH were stable, only two filters were tested in other environmental conditions.

3.3.3 Sublimation and dissolution of iodine molecules

Because iodine both sublimes and dissolves in water, the decrease in infectivity of viruses should be enhanced by increasing temperature and RH. To investigate the effects of iodine released from the iodine-treated filter and consequent accumulation in the impinger solution, clean air passing through the filter at two environmental conditions, HT/LRH and RT/MRH, was drawn into impingers containing a viral suspension of known concentration. The virus in the experimental impinger may lose its infectivity due to the operation of the impinger (*e.g.*, swirling and reaerosolization) and to toxicity of iodine molecules. Meanwhile, the infectivity of viruses in the control impinger will be affected only by the operation of the impinger. Thus, the loss of viral infectivity caused by stresses in the impinger was corrected by comparing the results of the control and the experimental impingers.

Sublimation of iodine molecules can be also verified by using sodium thiosulfate solution to quench the reaction of iodine molecules. The same experimental procedure described previously for iodine sublimation was followed except that the impinger medium was replaced by sodium thiosulfate solution. Thiosulfate anion stoichiometrically reduces iodine to iodide, which is not virucidal.

3.3.4 Infectivity of viruses on the filter

After 10 hrs of filtration experiments, the test filters were retrieved from the filter holder in the experimental system and subjected to the vortex mixer to investigate the infectivity of viruses collected on the filter. The filters were vortexed with sterile deionized water for a designated time (*i.e.*, 0, 1, 3, and 5 min) to investigate the optimal extraction time. The infectivity of viruses in the vortexing solution was assayed and the number of viruses (N_E) was determined as:

$$N_E = \frac{pfu}{10^{-n}} \times \frac{V_1}{V_2} \tag{5}$$

where *pfu* is plaque-forming units, V_1 is the volume of extraction fluid, V_2 is the volume of

original or diluted suspension assayed with host cells, and *n* is the dilution factor. The total infectivity of extracted viruses was calculated by averaging the results at all vortexing times because the number of extracted viruses at any designated vortexing time was found similar. To compare the result of the iodine-treated filter with the untreated filter we used survival fraction, which is defined as the ratio of the infectivity count in the extraction solution to the total viruses collected on the filter.

3.3.5 Effects of free iodine molecules

In an aqueous solution, the resin surfaces may release iodine molecules, which can inactivate viruses. This reaction raises concern that viruses can lose their infectivity in the extract solution due to the residual free iodine rather than on the filter. To investigate this possibility, the solution after vortexing a clean iodine-treated filter at a designated time (0, 1, 3, and 5 min) was inoculated with a virus suspension of known concentration. The infectivity of virus in each mixed suspension was analyzed after 15 minutes of exposure to the free iodine molecules in the suspension because it took around 15 minutes to perform the vortexing experiment. The concentration of iodine in the vortexing solution was determined by the DPD colorimetric method.

3.4 Condensation Nuclei Device

3.4.1 Conceptual implementation of in-line condensation nuclei device

The device used to achieve the condensation phenomenon comprised two essential components: a humidification section, in which the bioaerosols are introduced into a saturated water vapor atmosphere, and a condensation section, in which the atmosphere cools and becomes supersaturated with water vapor, which condenses on the biological nuclei. Figure 3.3 illustrates the schematics of this device.

A bioaerosol sample flow is introduced into the humidification stage, in which a heated pool of water is used to create a virtually saturated water vapor atmosphere (90–95% RH) at a slightly elevated temperature [35, 36]. After passing through the humidifier/saturator, the sample volume enters into the condenser, which consists simply of a cooled environment. This section lowers the water vapor temperature and creates a supersaturated condition. As the vapor becomes supersaturated, water condenses onto the bioaerosol nuclei, and the biological particle/droplet grows in diameter. The effective particle size is

significantly larger than the actual particle size of a bare airborne virus when the grown sample exits the condensational growth apparatus. The grown particle can now be collected with greater efficiency using traditional methods such as impingement or impaction.

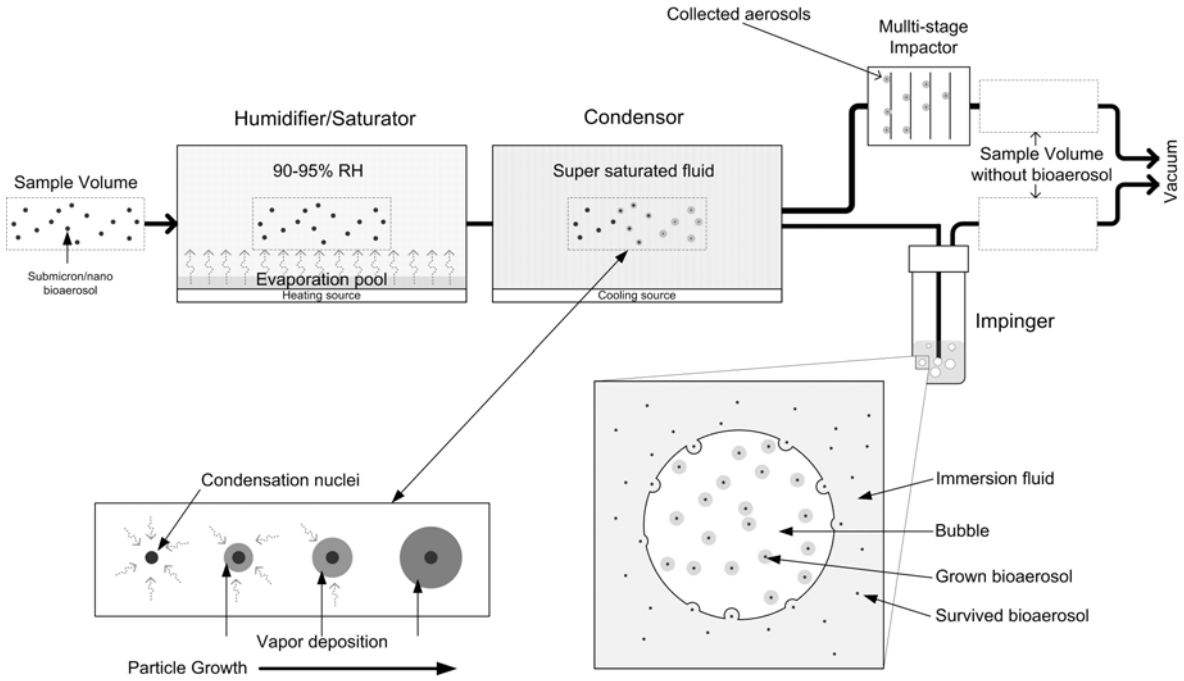


Figure 3.3. Viral aerosol grower conceptual schematic

3.4.2 Prototype design of condensation nuclei device

A prototype of the aforementioned device was developed for evaluation. The prototype design consists of two square aluminum tubes, aligned antiparallel along Peltier thermoelectric heat pumps, by which the upper tube is heated and the lower tube is cooled. The bioaerosol sample enters into the heated tube, in which it is saturated with water vapor, and then passes through the cooled tube, in which condensation occurs on the bioaerosol nuclei. Humidity at the exit of the heating tube is measured to ensure an ultimate humidity of at least 90% (relative). Four temperatures, at the beginning and ends of both tubes, are measured. A schematic diagram of the assembled device is shown in Figure 3.4 (a).

The length of both the heating and cooling tubes is a critical design factor for the proper operation of the condensation device. Since the heating and cooling sections had to be equal lengths to simplify design and construction, the cooling tube length was calculated

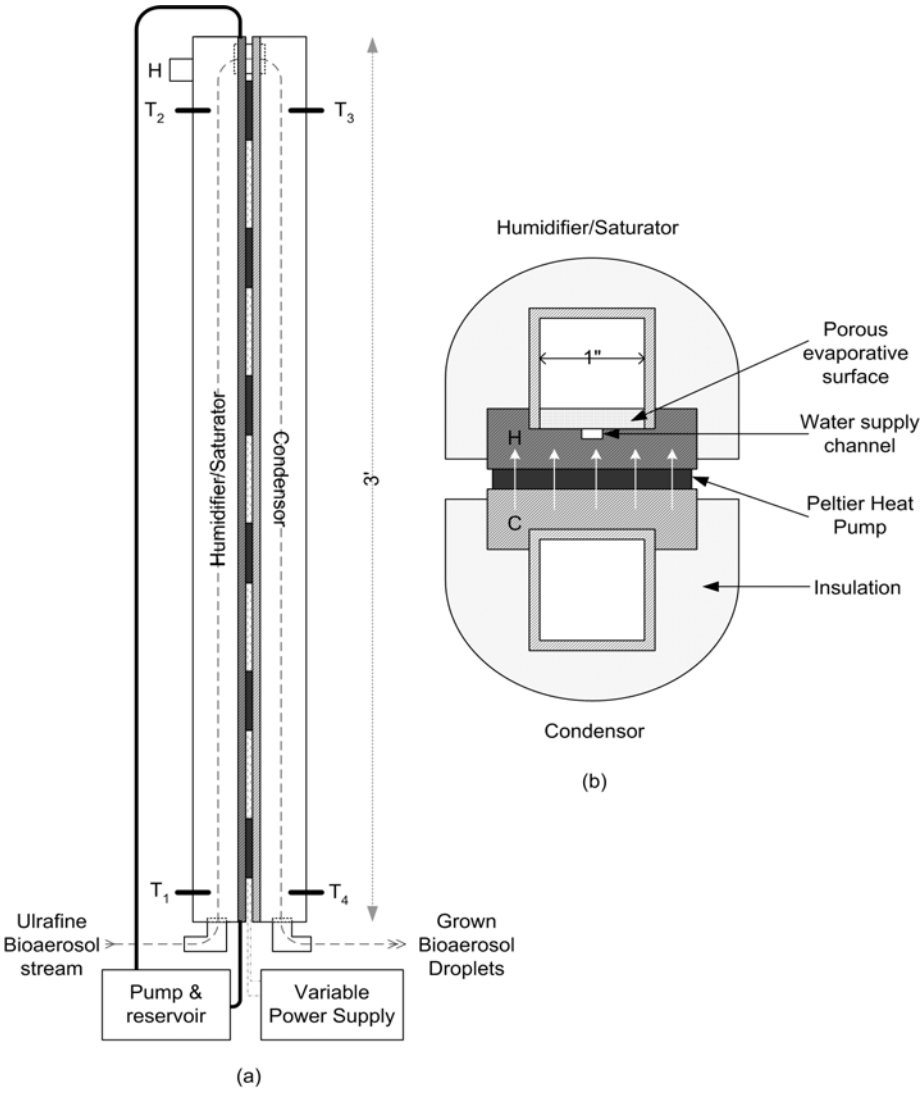


Figure 3.4. Schematic diagram of (a) viral aerosol grower prototype and (b) also in cross-sectional view

under the assumption that it was the limiting factor for the length, as cooling is more difficult than heating by means of evaporation. For a square tube of 2.54 cm width, an air mass flow rate of 12 L/min, and constant surface temperature, the minimum length of tubing is given by [49]:

$$L = \frac{T_{LM}\,\dot{m} C_P}{D^2 h} \tag{6}$$

where T_{LM} is the log mean temperature of the entering (T_{in}) and exit air streams

(T_{out}) and the cooling tube surface (T_S) tepmeratures, defined as

$$T_{LM} = -\ln\left[\frac{T_S - T_{in}}{T_S - T_{out}}\right] \tag{7}$$

A list of nomenclature is given in Table 3.1.

Table 3.1. List of nomenclature

Symbol	Property	Units
L	Tube length	m
D	Tube diameter	m
H	Heat transfer coefficient	W/m^2 ºC
T_S	Tube surface temperature	ºC
T_{in}	Inlet air stream temperature	ºC
T_{out}	Outlet air stream temperature	ºC
$\dot{m}$	Mass flow rate of air	kg/s
C_p	Heat capacity of air	kJ/kg ºC
Nu	Nusselt number	-
k	Thermal conductivity of air	W/m ºC

The heat transfer coefficient of the air flow through the tubing is given by:

$$h = \frac{Nu\, k}{D} \tag{8}$$

where the Nusselt number (Nu) has been tabulated for square tubing as Nu = 3.66 under the assumptions of laminar, fully developed flow. The heat capacity and thermal conductivity for air are C_p = 1.01 kJ/kg-ºC and k = 263 W/m-ºC, respectively. For an entering air temperature stream of 40 ºC and a surface temperature of 10 ºC, the required length to cool the air stream to 25 ºC is estimated to be L = 0.92 m (3.01 ft).

The temperature difference between the hot and cold sections is a critical means of control for producing supersaturated conditions. Six 8-W Peltier junctions were evenly distributed between two aluminum bases, which were attached to the heating and cooling tubes. In the cross-sectional view shown in Figure 3.4(b), the Peltier heat pump produces a heat flux across two surfaces, drawing energy from the cooling tube and delivering it into

the heating tube. The bases helped to distribute the intermittent thermal flux from the heat pumps along the tubes. The base temperature was monitored by thermocouples in both the hot and cold bases and the tube surface was considered to be equal to the base temperature. The effective tube temperature differences were controlled by varying the amount of voltage and current supplied to the Peltier array.

Water vapor was delivered into the heating chamber by a porous, hydrophilic, evaporative material produced by Porex, Inc. As seen in Figure 3.4(b), a water channel embedded in the base of the heating tube supplies a flow of water into the porous strip, whence it then transpires and evaporates into the flowing stream above it. The water stream passing through the channel is part of a closed circuit of water flowing via a small pump and reservoir seen in Figure 3.4(a). External insulation is used to retain efficiency and effectiveness of the heating and cooling system. The grown bioaerosol droplets exit the system and can be sampled by any means of existing bioaerosol sampling, such as an impinger or impactor.

3.4.3 Evaluation of condensation nuclei device

The experimental setup to evaluate the improved collection method using the new device is shown in Figure 3.5. The initial aerosols used to challenge the unit were dispersions of sodium chloride. To produce these inert aerosols, sodium chloride was dissolved in water in the nebulizer reservoir. To determine the appropriate concentration of sodium chloride in the nebulizer to produce a challenging particle size, the following equation was used [37]:

$$d_p = d_d \left(F_v\right)^{1/3} \tag{9}$$

The equation states that aerosol particles of diameter d_p, can be produced based on the droplet diameter, d_d, produced by the nebulizer and the volume fraction, F_v, of solid material in solution. To obtain an aerosol size of approximately 300 nm, 3.5 g/L of sodium chloride was dissolved in the nebulizer solution, giving an *Fv* of approximately 0.0035. A six-jet Collison nebulizer (Model # CN25, BGI Inc.) with a flow rate of 12.5 Lpm was used and was assumed to generate an aerosol approximately 2 μm in size [37]. As this experiment was largely to assure proper construction and operation of the unit, the aerosol size was not as critical as it will be in future experiments.

The generated aerosols were dried in the subsequent diffusion dryer and became the

aerosols of representative size. The aerosols entered the condensational growth unit and then were sampled with an impinger. As this test serves only to confirmation that the system is operating, the aerosol sizes do not have to be the most challenging size. The impinger collection liquid was analyzed using an ion chromatography system (ICS-1500, Dionex Corp.) to determine the concentration of sodium. As a control to confirm the success of the unit's design and construction, the same experimental procedure was followed without utilizing the condensational growth apparatus, such that the flow still went through the unit although it was not operating.

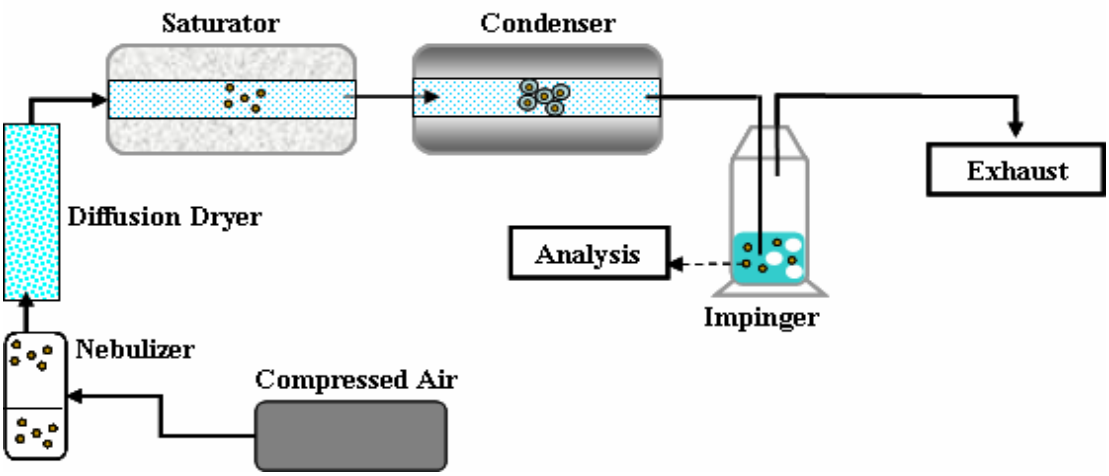


Figure 3.5. Experimental setup to evaluate the condensational growth unit

Sodium chloride was an appropriate choice to confirm the system's operation, but the unit was designed to be challenged with bioaerosols. For the bioaerosol test, the ability of the unit to improve sampling of airborne MS2 particles was evaluated using the same experimental setup as the sodium chloride experiment, with only the means of analysis differing. MS2 is an appropriate choice for use as a surrogate human pathogenic virus [45], and the size of the bacteriophage (27.5 nm) is a suitable challenge for the condensational growth unit. The nebulizer reservoir was prepared in the same manner explained in Section 3.3.1. The impinger collection liquid was analyzed using an enumeration technique for viable viruses.

Appendix B provides details regarding the preparation of the plaque assay medium. The liquid collected in the impingers was diluted to a suitable plate count, and the plaque- forming units were subsequently counted to determine how the condensational growth unit affected sampling of the airborne virus.

3.4.4 Reaerosolization of particles from the impinger

Figure 3.6 shows the experimental set-up for testing reaerosolization of particles from the impinger. A known concentration of viruses was placed in the collection medium and pure air was run through the impinger. The concentration of aerosol downstream of the impinger was measured by the SMPS. Rather than collecting aerosols in the impinger, this experimental design removes the variability associated with collection and provides a more accurate reaerosolization count.

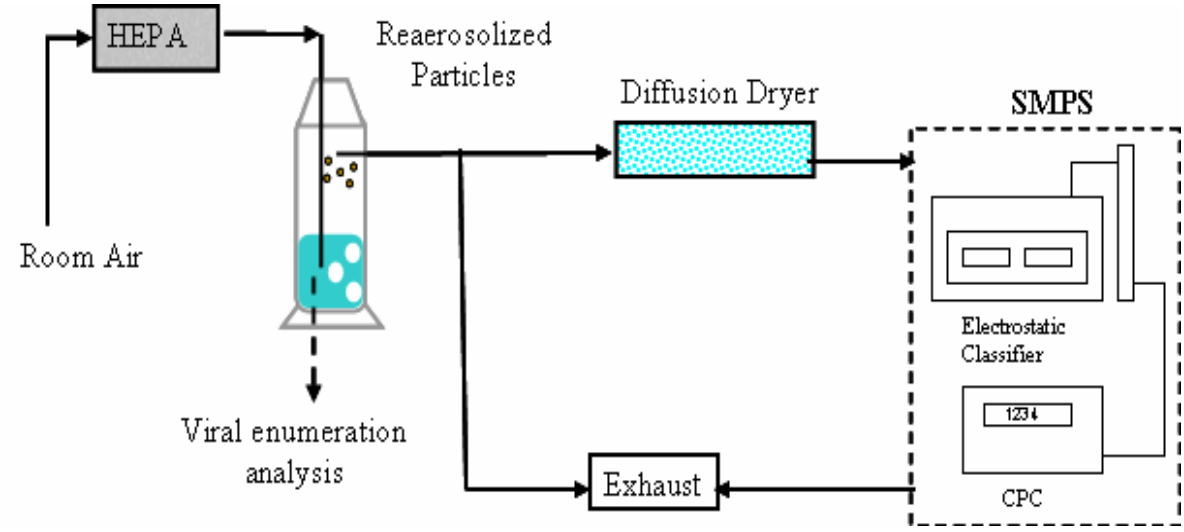


Figure 3.6. Experimental setup for the reaerosolization of particles from the impinger

To determine the effect of flow rate and impinger concentration on reaerosolization, a matrix was established with several different flow rates and concentrations as shown in Table 3.2. The matrix shows the direction in which the experiment progressed. A baseline test at 0 PFU/mL in the impinger collection liquid was run to confirm that the experimental setup was operating properly. The nebulizer in the baseline test hypothetically produces pure water droplets, in which case the diffusion dryer removes any moisture and the SMPS registers negligible aerosol particles. Twenty-five tests were run in total.

Table 3.2. Experimental plan to determine the effect of flow rate and concentration on reaerosolization.

Virus conc. (PFU/mL)	Flow rates (Lpm)				
	2	4	6	8	10
Baseline	B1	B2	B3	B4	B5
10^2	1	2	3	4	5
10^4	6	7	8	9	10
10^6	11	12	13	14	15
10^8	16	17	18	19	20

4 RESULTS AND DISCUSSIONS

The iodine-treated and untreated filters were evaluated for *B. subtilis* spores and MS2 bacteriophage in various environmental conditions. For comparison, the previous experimental results at RT/LRH reported in AFRL-ML-TY-TR-2007-4510 are also included.

4.1 Bacterial Spore Aerosols

The experiments were conducted at two environmental conditions, which are HT/LRH and RT/HRH. Raw data are presented in Appendix C.

4.1.1 Pressure drop

Pressure drop across the test filter was monitored using a Magnehelic gauge measuring 0–10 in. H_2O and was recorded every 20 minutes. Under the operating conditions, the initial pressure drop ranged from 6×10^2 to 9×10^2 Pa and was maintained throughout the entire experiment with almost negligible variation. There was no observable difference in pressure drop between the treated and untreated filters. To compare the pressure drop of the test filter with the glass fiber filter, pressure drag, *S*, was calculated. Pressure drag is the measure of the filter's aerodynamic resistance to air flow, which is defined as [50]:

$$S = \frac{\Delta P}{V_f} \tag{10}$$

where ΔP is the pressure drop across the filter and V_f is the face velocity. The pressure drag of the glass fiber filter is 4×10^4 Pa/(m/s), whereas that of the test filter has a much lower value, 6×10^3 Pa/(m/s). The calculation is presented in Appendix D.

4.1.2 Removal efficiency

Figure 4.1 shows the size distribution of the entering spores collected by the impactor. As shown, the majority of the entering spores were in the 0.65–2.1-μm range, indicating that they were predominantly singlets.

Regarding the removal efficiency, both iodine-treated and untreated filters had a high VRE (> 99.996 %) at RT/LRH, shown in Table 4.1, indicating that the PRE was so high that the VRE was not measurably affected by the iodine treatment on the filter. In

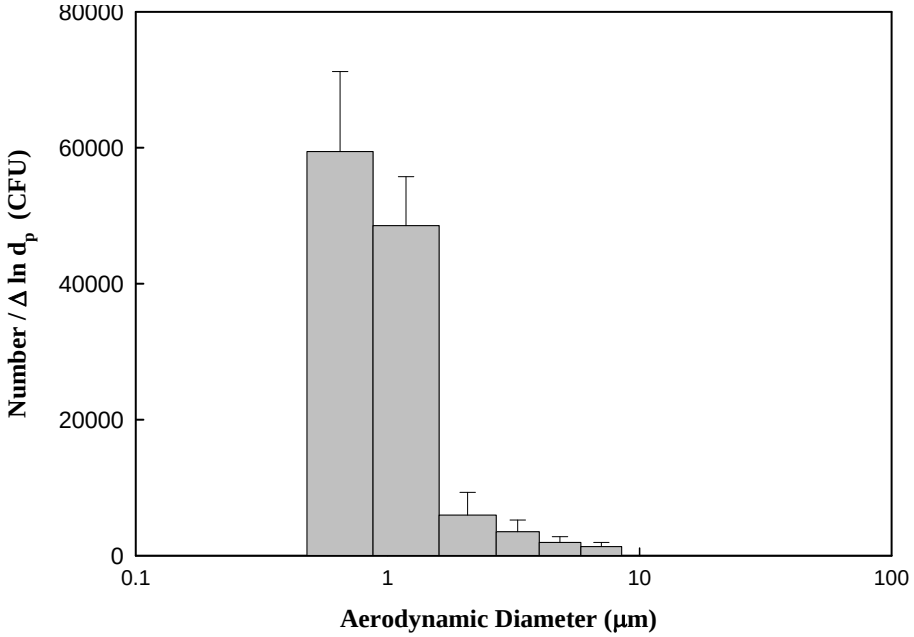


Figure 4.1. Particle size distribution of entering bacterial spores

other environmental conditions (*i.e.*, RT/HRH and HT/HRH), the iodine-treated filter also presented a high removal efficiency (> 99.998 %).

It should be noted that even when the filter did not show complete removal, in most cases, only one or two CFU penetration was detected downstream. There was no difference in any 2-hour interval, indicating that the performance did not deteriorate over time during the 10-hour or 4-hour experimental runs.

4.1.3 Survival fraction

To determine the viability of the collected spores, both iodine-treated and untreated filters were vortexed to extract spores from the filters. A slightly higher number of extracted spores from the untreated filter were enumerated than from the iodine-treatedfilter at RT/LRH. No increase of extracted spores from the test filters was observed as the vortexing time increased. Although both survival fractions were low, the survival fraction of the iodine-treated filter was lower than that of the untreated filter. At RT/HRH and HT/HRH, the survival fraction of the iodine-treated filter showed around one log unit higher value than that at RT/LRH. This higher survival fraction can possibly be explained by the loss of iodine from the filter because of the iodine's sublimation and dissolution. To

Table 4.1. Removal efficiency of the iodine-treated and untreated filter at various environmental conditions

Environmental conditions	Filter media	Trial no.*	Challenge (CFU)	Penetration (CFU)	Removal eff. (%)
RT/LRH †	Iodine-treated	1,5,7,9,10, 11,13,14,15	4.9×10^4–9.8×10^4	No	> 99.9980
		2	9.5×10^4	1	99.9989
		3	1.1×10^5	2	99.9981
		4	8.7×10^4	1	99.9988
		6	8.0×10^4	1	99.9988
		8	6.5×10^4	1	99.9985
		12	5.8×10^4	1	99.9983
	Untreated	1,2,4,6,7,9, 11,12,14	4.2×10^4–8.7×10^4	No	> 99.9976
		3	6.4×10^4	1	99.9984
		5	6.7×10^4	1	99.9985
		8	6.3×10^4	2	99.9968
		10	5.6×10^4	2	99.9965
		13	5.9×10^4	1	99.9983
		15	6.1×10^4	1	99.9984
RT/HRH ‡	Iodine-treated	1,2,3	7.3×10^4–8.1×10^4	No	> 99.9986
		4	8.0×10^4	1	99.9987
HT/HRH ‡	Iodine-treated	1,3,4	8.7×10^4–9.3×10^4	No	> 99.9989
		2	9.0×10^4	1	99.9989

*2-hr experiment per each trial, †15 trials, ‡4 trials

test this hypothesis, we measured the iodine concentration in the vortexing solution of the iodine-treated filter by the DPD colorimetric method. The values (mg I_2/L) of the iodine-treated filter tested at RT/HRH (0.40 ± 0.03) and HT/HRH (0.30 ± 0.03) were lower than that at RT/LRH (0.90 ± 0.03). However, we also note that *n* is small (*i.e.*, 2) and measurement uncertainty of survival fractions is large at both RT/HRH and HT/HRH.

To investigate the effect of free iodine residual in the vortexing solution on the survival fraction of the extracted spores, spores were inoculated into the solution after

vortexing a clean, iodine-treated filter at each designated vortexing time. As shown in Figure 4.2, the effect of the extracted iodine did not increase as vortexing time increased. The mean (± S.D) fraction of spores was 0.856 (± 0.014) and 1.01 (± 0.03) in the treated and untreated solution, respectively, indicating that the iodine extracted from the iodine-treated filter during vortexing decreased viability of spores in the solution by ~15%. Accordingly, the survival fraction of spores on the iodine-treated filter was corrected by this amount.

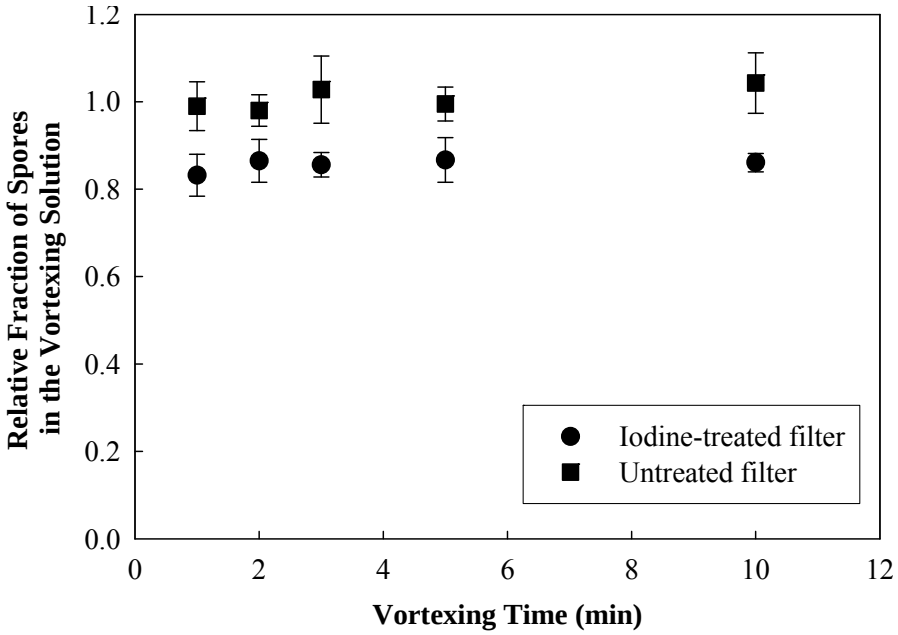


Figure 4.2. Relative fraction of spores in the vortexing solution of the clean iodine-treated and untreated filters

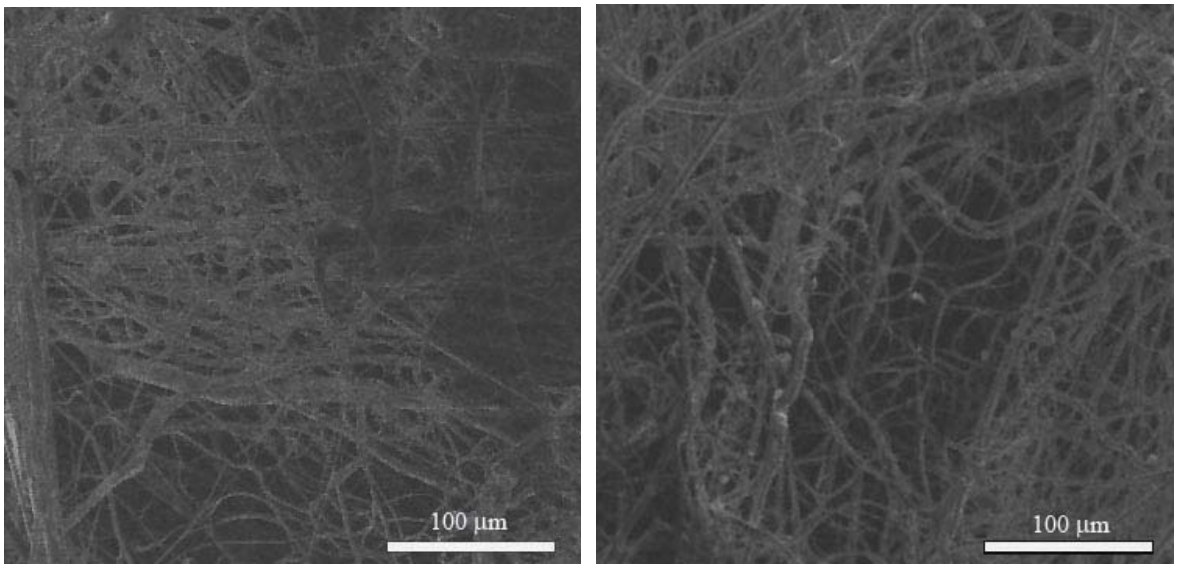
The effect of vortexing alone on viability of spores was also examined. A spore suspension of known concentration was vortexed for each designated time, after which viability of each was examined. The relative fraction obtained by dividing the number of viable spores after each vortexing time by that at zero vortexing time was calculated. The average (± S.D) fraction was 1.03 ± 0.15, showing that 10 mins of vortexing had negligible effect on viability of spores. Table 4.2 lists the corrected survival fraction considering only the effect of free iodine residual. The values are much lower than the result of a prior study, which reported 85% recovery using the vortexing method to extract *B. subtilis* spores from black polycarbonate filters [26]. The low survival fraction of our test filters shows that the vortexing method is not strong enough to completely extract collected spores from the

Table 4.2. Survival fraction of bacterial spores on both filters in various environmental conditions

Environmental conditions	Filter media	Average ± S.D
RT/LRH	Iodine-treated	$6.9\times10^{-4} \pm 1.6\times10^{-4}$
	Untreated	$2.5\times10^{-3} \pm 1.4\times10^{-3}$
RT/HRH	Iodine-treated	$5.1\times10^{-3} \pm 5.5\times10^{-3}$
HT/HRH	Iodine-treated	$8.3\times10^{-3} \pm 5.8\times10^{-3}$

filters. In other words, spores were trapped in the filter matrix very securely, resulting in inefficient extraction. From practical application perspectives, this demonstrates that the resin filter material without iodine treatment is still an effective medium to trap the microbial agents.

After vortexing, a tested and an unused iodine-treated filter were examined under a scanning electron microscope (SEM) (FESEM-6335F, JEOL) to look for spores not extracted from the filter. As shown in Figure 4.3, a few micron-sized particles remained in



(a) (b)

Figure 4.3. SEM images of the (a) unused iodine-treated filter and (b) tested iodine-treated filter at 100X.

the tested iodine-treated filter, whereas the unused filter was free of particles. These SEM images contribute support to the conclusion that inefficiency of removal by the vortexing method contributes to the low survival fraction of the filters.

4.2 Viral Aerosols Experiment

Viral experiments were conducted at two environmental conditions, RT/MRH and HT/LRH. The raw data are available in Appendix E.

4.2.1 Physical removal efficiency

The PRE of the test filters was measured by the SMPS, and was determined by comparing the PSDs of the aerosols entering and penetrating the test filters. In Figure 4.4, the PSD of the aerosols entering the test filters showed the mode at 30 nm, which is around the primary size of MS2 (27.5 nm). As a baseline, sterile deionized water without virus was aerosolized from the nebulizer and the PSD of that was measured. The average number of the PSD of sterile deionized water was 1.5×10^4 particles/cm^3, which can be a background noise. Therefore, the PSD of the aerosols observed above the data noise level—from 11.3 to 187.7 nm—was considered for the calculation. The PRE of the iodine-treated filter for this range was 32 ± 3 % in duplicate.

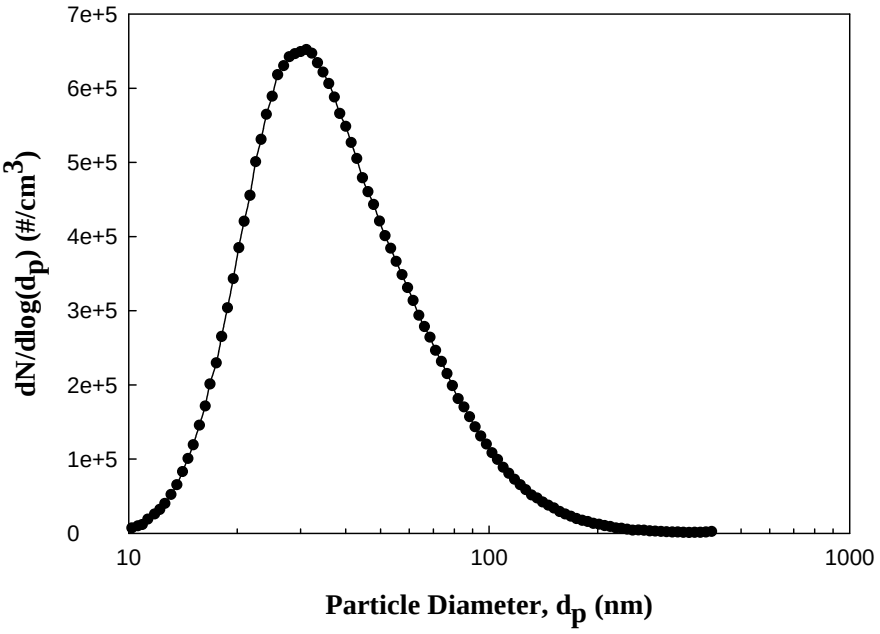


Figure 4.4. The number-based PSD of aerosols entering the test filter

4.2.2 Filter quality

The initial pressure drop of the test filters was around 0.2–0.3 in. H_2O and the variation in pressure drop during the entire experiment was negligible. To compare the iodine-treated filter with a glass fiber HEPA filter (Catalog # AP1504700, 47 mm, Millipore Co.), filter quality (q_F) [15] was calculated as:

$$q_F = \frac{\ln(1/P)}{\Delta P} \tag{11}$$

where ΔP is the pressure drop and P is the penetration. ΔP of the glass fiber filter at the face velocity of 5.3 cm/s was 2.1 kPa, whereas that of the iodine-treated filter at the same face velocity was 0.02 kPa. The PSD of virus entering and penetrating the filter was measured in the size range of 9.82–414.2 nm. The penetration by 30-nm particles, which is the mode of the PSD, was considered for the calculation. The filter quality of the iodine-treated filter was three times greater (16 kPa^{-1}) than that of the glass fiber filter (5 kPa^{-1}). The calculation is available in Appendix F.

4.2.3 Viable removal efficiency

The VRE of the test filters was calculated by analyzing the infectivity of viruses collected on both control and experimental impingers. The result is presented as an average of five 2-hr experimental runs. As shown in Table 4.3, iodine-treated and untreated filters presented a similar level of VRE at RT/LRH, 93.6 ± 1.2 % and 91.7 ± 0.9 %, respectively. This observation is inconsistent with expectation but the supply of media had been exhausted so it was not possible to repeat the experiment. At HT/LRH, the VRE of the iodine-treated filter exhibited the higher value (99.988 ± 0.018 %) expected from earlier results and the model proposed by Wu, Wander and coworkers, and that of the untreated filter (92.7 ± 1.9 %) was similar to the results at RT/LRH. If the observation of no enhancement of VRE at RT/LRH is correct, this increase in VRE of the iodine-treated filter must result from infectivity loss of viruses penetrating the filter caused by iodine that sublimed from the filter and was collected in the impinger downstream of the iodine-treated filter due to the sublimation of iodine at HT. Concentrations of available iodine shown in Table 4.6 after 10 minutes of direct extraction into water caused around 83 % attenuation of viability in impingers. The VRE of the filters tested at RT/MRH was similar to the results at HT/LRH, 99.763 ± 0.005% and 92.3 ± 1.5 % for the iodine-treated and untreated filters,

Table 4.3. Removal efficiency of the iodine-treated and untreated filters in various environmental conditions

Environmental conditions	Filter media		Virus Concentration (PFU/mL)*		Removal eff. (%)*
			Challenge	Penetration	
RT/LRH	Iodine-treated	No.1	$7.8\times10^{4}\pm5.9\times10^{4}$	$3.7\times10^{3}\pm2.8\times10^{3}$	94.2±2.8
		No.2	$5.7\times10^{4}\pm3.2\times10^{4}$	$2.8\times10^{3}\pm1.2\times10^{3}$	94.4±2.2
		No.3	$6.9\times10^{4}\pm5.6\times10^{4}$	$5.5\times10^{3}\pm4.8\times10^{3}$	92.2±2.4
	Untreated	No.1	$6.3\times10^{4}\pm5.6\times10^{4}$	$5.0\times10^{3}\pm4.4\times10^{3}$	92.4±1.8
		No.2	$3.7\times10^{4}\pm1.2\times10^{4}$	$3.3\times10^{3}\pm1.1\times10^{3}$	90.7±2.2
		No.3	$4.4\times10^{4}\pm1.2\times10^{4}$	$3.4\times10^{3}\pm1.2\times10^{3}$	92.1±2.2
HT/LRH	Iodine-treated	No.1	$1.4\times10^{5}\pm7.0\times10^{4}$	N.D†	> 99.99995
		No.2	$3.0\times10^{4}\pm2.5\times10^{4}$	$3.2\times10^{0}\pm2.4\times10^{0}$	99.98±0.05
	Untreated	No.1	$3.3\times10^{5}\pm1.5\times10^{5}$	$1.6\times10^{4}\pm6.9\times10^{3}$	94.0±3.8
		No.2	$9.6\times10^{4}\pm3.0\times10^{4}$	$7.2\times10^{3}\pm2.7\times10^{3}$	91.4±4.8
RT/MRH	Iodine-treated	No.1	$2.4\times10^{4}\pm1.8\times10^{4}$	$6.7\times10^{1}\pm6.9\times10^{1}$	99.8±0.3
		No.2	$7.6\times10^{3}\pm3.2\times10^{3}$	$4.2\times10^{0}\pm8.8\times10^{0}$	99.8±0.8
	Untreated	No.1	$2.3\times10^{5}\pm2.4\times10^{5}$	$1.4\times10^{4}\pm1.3\times10^{4}$	93.4±2.1
		No.2	$1.0\times10^{5}\pm3.8\times10^{4}$	$8.9\times10^{3}\pm3.5\times10^{3}$	91.3±2.0

* The average(± S.D) of five 2-hr trials, † Not detected.

respectively. An even less probable enormous enhancement in the rate of dissolution or extraction of iodine molecules from the iodine-treated filter at medium RH would have to be invoked to explain the two-log increase in iodine concentration in the impingers caused by a relatively small change in RH. So, we are left to conclude that the observation of no enhancement of VRE by the iodine treatment requires more thorough evaluation.

It should be noted that PRE was measured for ultrafine particles (11.3–187.7 nm), whereas VRE was measured over the entire particle size range generated from the nebulizer. Even if the PRE for the entire particle size range is calculated from particle counter data, its value is still much lower than the VRE for two possible reasons—low collection efficiency of the impinger for ultrafine particles, and virus aggregation and lower counts of viable virus in smaller particles than in bigger particles. These are discussed below.

1. Low collection efficiency of impinger for ultrafine particles

The dominant collection mechanism of the impinger is impaction, which improves in efficiency as the aerosol size increases. Hogan et al.[14], who studied the application of an impinger for viral aerosol collection, concluded that none of the samplers tested—including the Biosampler, AGI-30, and frit bubbler—are effective for collection of ultrafine and submicron virus particles. In the test system, most viruses penetrating the test filters experienced low collection efficiency in the impingers because the particles were in the ultrafine range. Larger aerosols could be more efficiently collected in the control impingers whereas in the experimental stream the filter intercepted the larger aerosols. The low collection of ultrafine aerosols in experimental impingers combined with the high collection of larger aerosols in control impingers thus exaggerates the measured increase in VREs over PREs by the test filter.

2. Virus aggregation and lower count of viable virus for ultrafine particles

Another factor that may contribute to overestimation of the contribution of iodine chemistry to VRE derives from the viable virus count in a particle of given size. The particle counter measures the virus aggregate as one particle, but it can be assayed as several viruses after collection in the impinger because of dispersion in the collection medium. The number of viable viruses in a big particle is significantly higher than that in an ultrafine particle; thus the collection of larger particles in the impingers will contribute to the infectivity results greater than that of ultrafine particles. This assumption is supported by a prior study [14], which reported that a larger particle from MS2 suspension has a higher possibility of containing a viable virus. One avenue to decrease this complication is to increase the particle sizes by condensational growth and thus their collection efficiency.

4.2.4 Sublimation and dissolution of iodine

The effect of iodine sublimation at high temperature and the dissolution of iodine molecules at medium RH were investigated by using the impingers containing a known concentration of virus suspension. The infectivity of viruses in both control and experimental impinger was analyzed every 30 mins and the virus concentration in the impingers was varied over a 3-log range (10^3, 10^4, 10^5 PFU). As shown in Table 4.4, no surviving virus was detected in the experimental impinger until > 10^4 PFU was added to the impinger, indicating the effect of released iodine on the infectivity of viruses collected in the experi-

Table 4.4. Survival of MS2 from the sublimation and dissolution of iodine in the impingers

Environmental conditions	Virus count in the impingers (PFU)		Relative fraction‡
	Control	Experimental	
HT*	6.2×10^{3}	0	0
	6.3×10^{3}	0	0
	4.7×10^{4}	62	1.3×10^{-3}
	5.9×10^{4}	107	1.8×10^{-3}
	1.8×10^{5}	349	2.0×10^{-3}
	2.3×10^{5}	644	2.7×10^{-3}
MRH†	4.4×10^{3}	0	0
	7.9×10^{3}	0	0
	6.0×10^{4}	35	5.8×10^{-4}
	7.0×10^{4}	62	8.8×10^{-4}
	2.8×10^{5}	528	1.9×10^{-3}
	3.0×10^{5}	589	2.0×10^{-3}

*For iodine sublimation, †For iodine dissolution, ‡PFU in experimental impinger was divided by PFU in control impinger

mental impinger. The same phenomenon was observed in the experimental impinger tested at medium RH. As the virus concentration in the impinger increased, the number of viruses surviving also increased. This may be due to a shielding effect when viruses are aggregated or encased or to exhaustion of available iodine. However, a study on the survival curve of viral particles in the aqueous suspension irradiated with ultraviolet light demonstrated that survival of viruses depends strongly on the degree of aggregation of the viral particles [52].

To verify sublimation of iodine molecules, the infectivity of viruses in both impingers containing virus suspension in sodium thiosulfate solution was analyzed. As shown in Table 4.5, the relative fraction was much higher than the value shown in Table 4.4 at the same level of concentration in the control, demonstrating the effect of iodine molecules on the infectivity of viruses at HT. Most viruses suspended in the thiosulfate solution survived in the experimental impinger due to the quenching of the iodine molecules released from the iodine-treated filter by thiosulfate, either at the surface of capturing microbes or in solution.

Table 4.5. Relative fraction of MS2 in the impingers containing thiosulfate suspension

Environmental conditions	Virus count in the impingers (PFU)		Relative fraction
	Control	Experimental	
HT	3.9×10^4	2.6×10^4	0.67
	4.7×10^4	3.4×10^4	0.71

4.2.5 Survival fraction and effects of free iodine molecules

The infectivity of viruses collected on the filter is expressed as the survival fraction (C_E/C_C, C_E: Extracted MS2 from the filter, C_C: MS2 collected on the filter). The infectivity of viruses did not decrease during 10 mins of vortexing, indicating that the effect of vortexing on the viruses was negligible. In the free iodine effect experiment, the average iodine concentration in the vortexing solution at all vortexing times, measured by the DPD colorimetric method, was around 1.0 mg/L I_2 as presented in Table 4.6. Some iodine was released from the iodine-treated filter before the start of vortexing, designated as "0" vortexing time. No further increase of iodine extraction from the filter by increasing vortexing time was observed. The infectivity of viruses mixed with the vortexing solution of a clean iodine-treated filter at each designated vortexing time was analyzed, and expressed as relative fraction (C_S/C_I, C_S: Survived MS2, C_I: Initial MS2 in the suspension). The average value of the relative fraction, 0.17 (*i.e.*, 83 % attenuation), was used to correct the survival fraction of MS2 extracted from the iodine-treated filter.

Table 4.6. Iodine concentration (mg I_2/L)* in the vortexing solution at each vortexing time

Filter media	Vortexing time (min)			
	0	1	5	10
Iodine-treated	0.62 ± 0.11	0.98 ± 0.04	0.91 ± 0.13	0.98 ± 0.08

* The average measurement in triplicate

In the calculation of the survival fraction at HT/LRH and RT/MRH, C_C was determined from the VRE of the untreated filter medium based on the assumption that both iodine-treated and untreated filters have a similar PRE. This assumption was derived from the experiment of sublimation and dissolution of iodine, which verified that higher VRE of

the iodine-treated filter at HT/LRH and RT/MRH resulted from disinfection by environmental iodine. Table 4.7 presents observed and corrected values of the survival fraction of MS2 captured by treated and control media. As shown, no significant difference in the survival fraction between iodine-treated and untreated filters was observed at the same environmental conditions. However, both iodine-treated and untreated filters tested at MRH showed the lowest value among the survival fractions. A possible reason for this fact is the sensitivity of MS2 to the MRH [48, 53].

Table 4.7. Survival fraction of MS2 on the iodine-treated and untreated filters at various environmental conditions

Environmental conditions	Filter media	Average ± S.D		Iodine in vortexed solution (mg/L)
		Observed	Corrected	
RT/LRH	Iodine-treated	$3.4\times10^{-3}\pm1.4\times10^{-3}$	$2.0\times10^{-2}\pm8.4\times10^{-3}$	0.93±0.01
	Untreated	$3.6\times10^{-2}\pm4.3\times10^{-2}$		-
HT/LRH	Iodine-treated	$3.3\times10^{-3}\pm2.0\times10^{-3}$	$2.0\times10^{-2}\pm1.2\times10^{-2}$	0.575±0.007
	Untreated	$3.3\times10^{-2}\pm2.7\times10^{-2}$		-
RT/MRH	Iodine-treated	$1.2\times10^{-3}\pm5.0\times10^{-4}$	$6.9\times10^{-3}\pm2.9\times10^{-3}$	0.76±0.06
	Untreated	$5.5\times10^{-3}\pm9.2\times10^{-4}$		-

Because it is implausible to assume that iodine would exert a protective effect, the observation that the corrected survival fractions of both filters were the same while the uncorrected values were lower for the treated filters is more likely an artifact of the correction process than an indication that inactivation of viruses in the impinger was a major factor in this experiment. The lower measured concentration (mg I_2/L) of the iodine-treated filter tested at HT/LRH and the smaller increase at HT/MRH than that at RT/LRH might be caused by increased loss of iodine from the iodine-treated filter by enhanced rates of sublimation and dissolution or extraction of iodine molecules at the respective environmental conditions.

4.3 Condensation Nuclei Device

The experiment to confirm the design and construction of the condensational growth unit was conducted using sodium chloride and MS2 bacteriophage aerosols, and only preliminary results are available.

4.3.1 Testing of condensation growth unit

Table 4.8 shows the collection of sodium chloride in the impinger after passage through the condensational growth unit. The system remained the same throughout the entire test series—the unit was still in place even when it was off—to maintain consistency in the system. The increased concentration of sodium chloride in the impinger when the unit is turned on validates the effectiveness of the design and construction of the condensation growth unit.

Table 4.8. Collection of sodium chloride in the impinger with the operation of the condensational growth unit

Condensational growth unit status	NaCl in the impinger (ppm)
Off	10.8
On	13.5
On	16.0
Off	13.8

The subsequent testing utilized the same system setup as the sodium chloride tests but challenged with airborne MS2 viruses instead. This phase of testing was recently initiated, and preliminary results are displayed in Table 4.9.

The preliminary results from the airborne MS2 virus sampling with the condensational growth unit in operation are promising, nearly doubling the collection of viruses with an 87% increase. Future experiments will continue to involve the use of viral aerosols.

Table 4.9. Preliminary results from viral aerosols sampling using the condensational growth unit

Sampling time (30 min)	Condensational growth unit status	Virus count (PFU)	Mean virus count (PFU)
Control	Off	76,000	78,000
Control	Off	80,000	
Experiment	On	166,000	146,000
Experiment	On	131,000	
Experiment	On	143,000	

4.3.2 Reaerosolization of particles from the impinger

Dependence of the reaerosolization rate of MS2 particles on flow rate and a known MS2 concentration in the impinger was evaluated using a diffusion dryer followed by a differential mobility analyzer to quantify particles downstream of the impinger. Five tests at each scenario were run for a 15-min period. Results in Figure 4.5 show that increasing air- flow significantly increases the number of virus particles reaerosolized. The increase in reaerosolized particles peaks near 30 nm, which is close to the size of MS2 (27.5 nm).

Regarding the effect of impinger concentration on the reaerosolization shown in Figure 4.6, increased concentration does not necessarily lead to an increase in reaerosolization. Rather, the count of reaerosolized particles increases as concentration increases until it reaches a concentration of approximately 10^6 PFU/mL, at which point the reaerosolized count started to decrease. A possible explanation for this observation is re-aggregation of virus particles at high concentrations within the collection liquid, as a means of protection because of the stressful conditions inside of the impinger. Future testing will explore this observation.

Figure 4.7 provides a summary of the results in the form of the total number concentration of aerosols exiting the impinger, as determined by the SMPS. From this experiment, it is clear that reaerosolization is expected to compete with gains from the new condensational growth unit whenever an impinger is used to collect ultrafine bioaerosols.

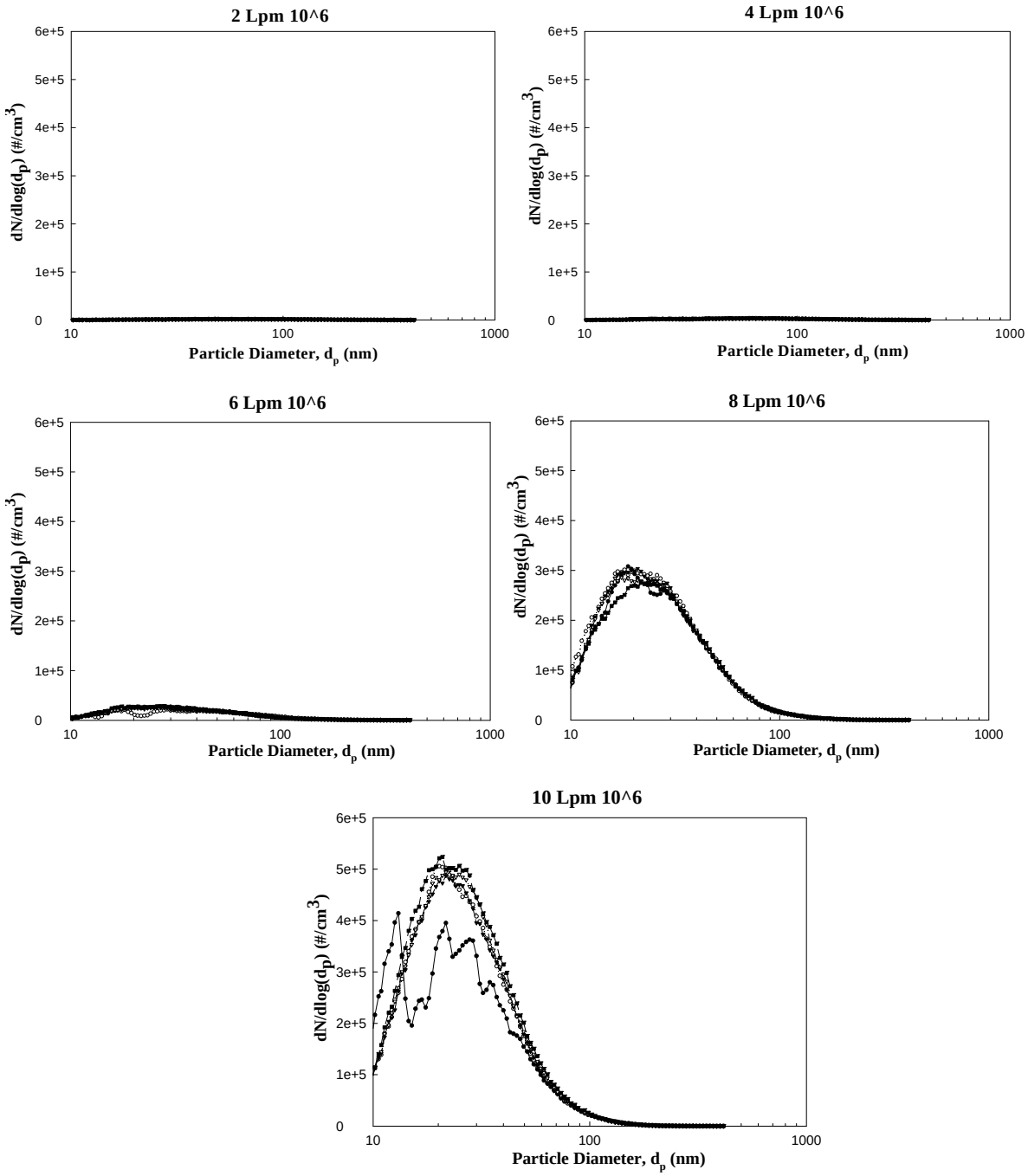


Figure 4.5. Reaerosolization of particles as a function of flow rate

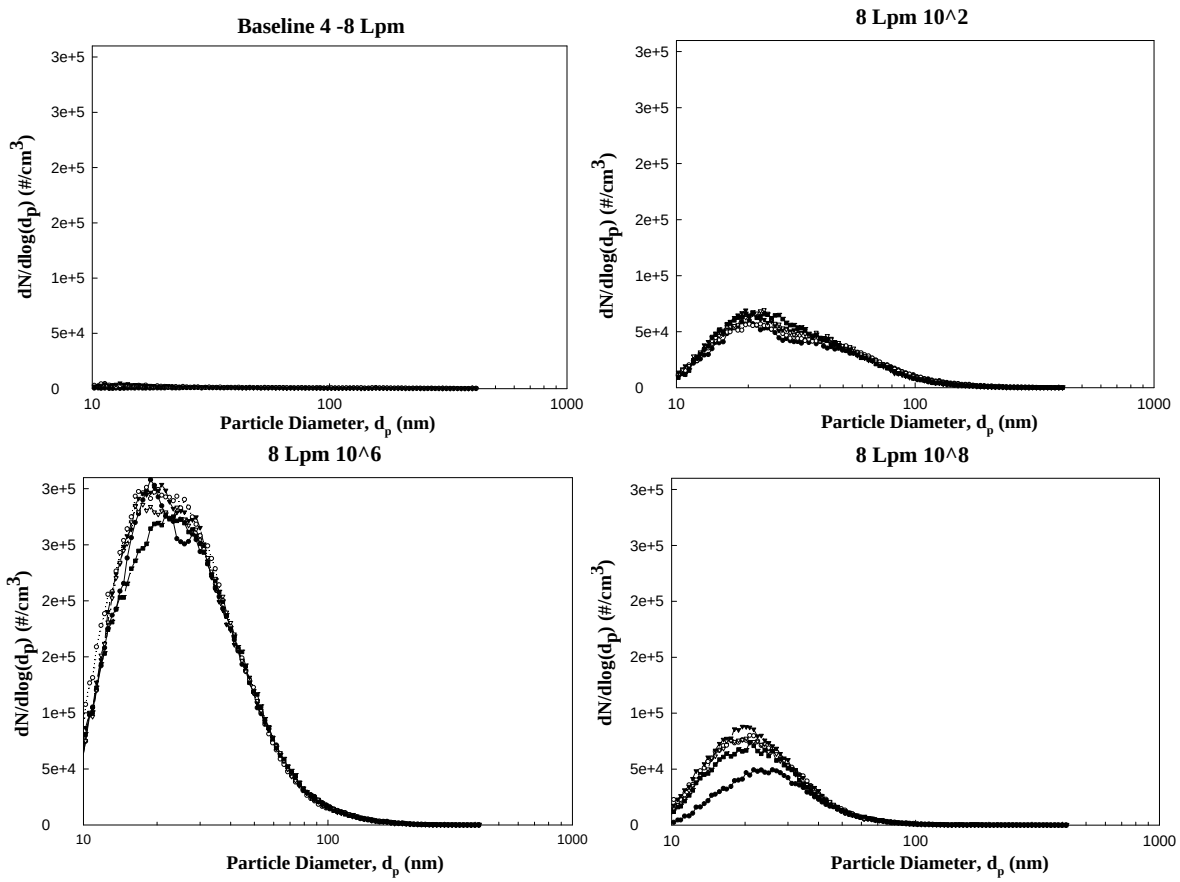


Figure 4.6. Reaerosolization of particles as a function of collection liquid concentration

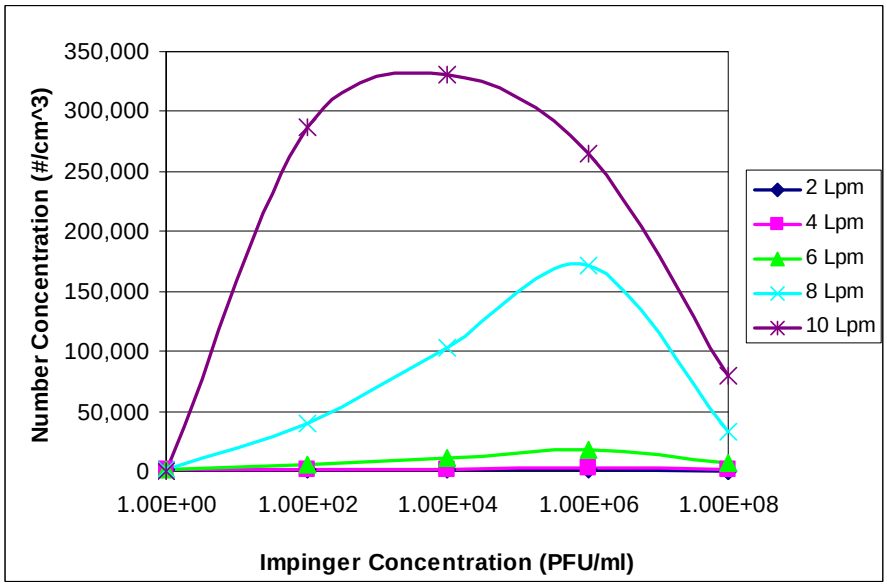


Figure 4.7. Reaerosolization as a function of flow rate and impinger concentration

5 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Both iodine-treated and untreated filters exhibited high VRE (> 99.996%) for bacterial spore aerosols (0.65 ~ 2.1 μm) in various environmental conditions. This great performance of test filters did not deteriorate over the experimental duration (*i.e.*, 10 hr or 4 hr). Initial pressure drop of the test filters ranged from 6×10^2 to 9×10^2 Pa and the variation in pressure drop was negligible. Pressure drag of the test filter (6×10^3 Pa/(m/s)) was much lower than that of the glass fiber HEPA filter (4×10^4 Pa/(m/s)). Viability of spores collected on the filter was investigated by extracting them from the filter and presented as the survival fraction (C_E/C_C, where C_E: spores extracted from the filter and C_C: spores collected on the filter). A higher survival fraction of untreated filter than that of the treated filter was reported at RT/LRH. However, the survival fraction of treated filter shown at RT/HRH and HT/HRH was similar to that of untreated filter tested at RT/LRH, indicating negligible effect of iodine treatment. Some loss of iodine was expected due to sublimation and dissolution at HT and HRH, and evidence for this was seen in the iodine analysis in the vortexing solution.

For the viral aerosol experiment, new filter media different from those used in the bacterial spore experiment were supplied by AFRL. The test filters (16 kPa^{-1}) exhibited greater filter quality than the glass fiber HEPA filter ((5 kPa^{-1}). Both iodine-treated and untreated filters exhibited similar VRE at RT/LRH; however, because higher VRE of the iodine-treated filter than that of untreated filter was shown at HT/LRH and RT/MRH as in the bacterial and earlier studies, this is suspected to have been an experimental error. The sublimation and dissolution of iodine released from the treated filter at HT and MRH may also affect the infectivity of virus. The survival fraction of treated and untreated filters was similar to each other at each set of environmental condition. Due to the sensitivity of MS2 to mid-range RH, the lowest survival fraction of both treated and untreated filters was observed at RT/MRH. The insignificant effect of iodine on the infectivity of MS2 can be explained by the shielding effect of aggregated/encased MS2 particles collected on the filter.

The condensation nuclei device designed to improve the collection of viral aerosols increased collection efficiency by ~ 48 % for NaCl testing aerosols and > 87% for MS2 aerosols. Further experiments are needed to better characterize the effect on bioaerosols in the ultrafine range. Reaerosolization from impingers was also investigated and found to

increase as the flow rate increased. However, counts of reaerosolized particles increased as concentration increased until the virus concentration reached 10^6 and 10^8 PFU/mL, above which it started to decrease.

5.2 Conclusions

Several conclusions may be drawn from the results of bacterial spore aerosol experiments:

(1) Iodine-treated filter presented high VRE for bacterial spores with a low pressure drop compared to HEPA filter in various environmental conditions.

(2) The bacterial spores collected on the iodine-treated filter were inactivated to certain degree compared to the untreated filter.

(3) Loss of iodine due to iodine's sublimation and dissolution might limit the effect of iodine on the viability of spores on the filter.

(4) Re-entrainment of microbes from the filter is limited, so the resin filter without iodine treatment also can effectively trap bacterial spores.

Several conclusions may likewise be drawn from the viral aerosol experiments:

(1) The filter media used for virus experiments showed a lower apparent VRE than that of bacterial spore experiments. Three possible reasons for the lower VRE are the size of the test aerosol, characteristics of the filter and discrimination against the smaller particles in the impinger collection fluid.

(2) Both iodine-treated and untreated filters inexplicably exhibited a similar VRE at RT/LRH. At RT/MRH and HT/LRH, the iodine-treated filter showed the expected higher VRE than that of the untreated filter. The released iodine molecules from the filter significantly contribute to but do not dominate the VRE.

(3) Insignificant difference was observed between the survival fraction of viruses on iodine-treated and untreated filters at the same environmental condition, indicating a negligible contribution of iodine treatment to death of viruses in air.

(4) Aggregation/encasement of microorganisms can gradually erode the efficacy of an antimicrobial filter because shielding them from the antimicrobial agent[s] preserves the viability of captured microorganisms. This possibility makes air filters a potential source of

microbial contamination.

And three conclusions may be drawn from the experiments with the condensation nuclei device:

(1) The increased collection efficiency of the impinger was demonstrated for sodium chloride and MS2 aerosols by operating the condensation nuclei device.

(2) Increasing the impinger flow rate significantly increases the reaerosolization of virus particles from the impinger.

(3) Increased reaerosolization correlated with an increase of virus concentration in the impinger only up to a moderate concentration; reaerosolization of particles increased until the concentration in the impinger reached approximately 10^6 PFU/mL. Re-aggregation of particles in the liquid at high concentration may contribute to this phenomenon.

5.3 Recommendations

Further research is needed to ensure the effectiveness of iodine-treated biocidal filter in real applications. In the operation of filtration systems as well as respirators, filters collect all varieties of aerosols including mineral dust particles or particles generated from combustion sources as well as bioaerosols. The presence of these particles may hinder the exertion of biocidal effect by interaction with the active site of filters designed to react with microorganisms. Furthermore, these substances can serve as nutrients for the growth of collected microorganisms resulting in the inhalation of bioaerosols from re-entrainment. Therefore, the potential for development of diminished disinfection capacity of iodine-treated filter media under these conditions should be investigated.

Certain experimental methodology was recommended for future research. The adoption of sodium thiosulfate solution as a collection medium of the impingers will exclude the effect of iodine on the infectivity of viruses at high temperature and increased RH. The thiosulfate anion will stoichiometrically react with iodine and reduce it to iodide, which is not virucidal [44].

Regarding the use of the condensation nuclei device, experiments with bioaerosols of better defined size range are needed to confirm that the system can achieve results similar to existing system used to improve collection efficiency of ultrafine particles [34]. However, prior to testing the unit with bioaerosols, the use of inert particles (*e.g.*,

polystyrene latex particles of approximately 30 nm) will eliminate the issues of viability relating to bioaerosols. Once that experiment has achieved results similar to that found in prior research, the next step will be to utilize bioaerosols and bring the variability associated with microorganisms back into the system. A non-pathogenic nanoparticle of diameter 27.5 nm [54], MS2 bacteriophage can be used for aerosol testing. The SMPS will provide physical collection efficiencies, while viral enumeration of the impinger collection liquid will provide viable collection efficiencies.

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Appendix A. Spore Production and Purification Procedures

The African violet method (African violet soil, 77.0 g; Na_2CO_3, 0.2 g; distilled water, 200.0 mL) suggested by The American Type Culture Collection [55] was chosen for sporulation. The nutrient agar was made of 25% soil extract autoclaved and mixed with 75% sterile distilled water. *B. subtilis* was inoculated in the African violet agar slant and incubated at 36 °C for one week to produce spores 0.8~1.2 μm in length, of either spherical or ellipsoidal shape [56]. After spore production, bacterial growth was harvested into 2 mL sterile distilled water and poured into a sterile glass tube. The glass tube and spore suspension were heated in a water bath at 80 °C for 30 mins to kill vegetative cells. The cooled spore suspension was diluted with 5 mL sterile distilled water and centrifuged at 3500 rpm for 5 mins. Separated cell debris was then removed in the supernatant. This process was repeated twice more and the spores were resuspended in 5 mL sterile distilled water. The purified spore suspension was stored in a refrigerator at 4 °C before experimentation. Microscopic observation of the spore suspension after applying malachite green spore-staining technique [57] showed the majority to be endospores with minute amounts of cell debris.

Appendix B. Procedures for Preparing Plaque Assay Media

- **MS2 Media**

With gentle mixing, 1.0 g tryptone, 0.1 g yeast extract, 0.1 g D-glucose, 0.8 g NaCl, and 0.022 g $CaCl_2$ wre added to a total volume of 100 mL of distilled water in a 250-mL flask. The mixed medium was autoclaved at 121 °C for 30 mins.

- **MS2 Agar Media**

With gentle mixing, 3.0 g tryptone, 0.3 g yeast extract, 0.3 g D-glucose, 2.4 g NaCl, 0.066 g $CaCl_2$, and 0.3 g of Bacto-agar were added to a total volume of 300 mL of distilled water in a 500-mL flask. The mixed agar was autoclaved at 121 °C for 30 mins.

- **1XPBS dilution tube**

KH_2PO_4 (1.8 g), 15.2 g K_2HPO_4, and 85 g NaCl were added to 1 L of distilled water to make 10XPBS. 1XPBS was prepared by diluting 10XPBS in distilled water. Aliquots (9 mL) of 1XPBS were dispensed into 16 × 150 mm test tubes and autoclaved at 121 °C for 30 min.

Appendix C. Raw Data of Bacterial Spore Experiments

Iodine-treated filter 1 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor Stage	CFU
20	3.4	34	0	1	1248
40	3.4	32	0	2	1812
60	3.4	32	0	3	3096
80	3.5	32	0	4	5712
100	3.5	33	0	5	28068
120	3.5	32	0	6	58248
Experiment 2.		Room Temperature: 23±2 °C			
20	3.5	36	0	1	888
40	3.6	32	0	2	1860
60	3.5	32	1	3	2724
80	3.5	33	0	4	4920
100	3.5	33	0	5	35616
120	3.5	33	0	6	48552
Experiment 3.		Room Temperature: 23±2 °C			
20	3.4	38	0	1	960
40	3.4	33	1	2	1572
60	3.5	32	1	3	2904
80	3.4	33	0	4	11628
100	3.4	33	0	5	44580
120	3.6	32	0	6	44304
Experiment 4.		Room Temperature: 23±2 °C			
20	3.4	38	1	1	792
40	3.4	33	0	2	936
60	3.5	32	0	3	1884
80	3.4	33	0	4	3024
100	3.4	33	0	5	44580
120	3.6	32	0	6	35616
Experiment 5		Room Temperature: 23±2 °C			
20	3.6	35	0	1	408
40	3.6	34	0	2	936
60	3.6	32	0	3	1428
80	3.7	32	0	4	3228
100	3.7	33	0	5	42960
120	3.6	34	0	6	29520

* CFU is the number of microorganisms normalized to 120 minutes.

Iodine-treated filter 2 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.0	34	0	1	552
40	3.0	33	1	2	612
60	3.0	33	0	3	1212
80	3.0	33	0	4	2388
100	3.0	33	0	5	31344
120	3.2	33	0	6	44016
Experiment 2		Room Temperature: 23±2 °C			
20	3.0	40	0	1	792
40	3.0	38	0	2	948
60	3.0	34	0	3	1644
80	3.0	34	0	4	2616
100	3.0	33	0	5	33372
120	3.2	33	0	6	25884
Experiment 3		Room Temperature: 23±2 °C			
20	3.0	38	0	1	360
40	3.1	36	0	2	432
60	3.0	33	0	3	1008
80	3.0	32	1	4	2064
100	3.0	32	0	5	30192
120	3.0	32	0	6	31200
Experiment 4		Room Temperature: 23±2 °C			
20	3.0	39	0	1	264
40	3.0	38	0	2	564
60	3.0	35	0	3	780
80	3.0	33	0	4	2088
100	3.0	33	0	5	30144
120	3.1	33	0	6	25944
Experiment 5		Room Temperature: 23±2 °C			
20	3.0	39	0	1	516
40	3.0	37	0	2	900
60	3.0	34	0	3	1200
80	3.0	33	0	4	2484
100	3.0	32	0	5	34548
120	3.3	32	0	6	31752

Iodine-treated filter 3 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.3	39	0	1	204
40	3.3	34	0	2	420
60	3.4	33	0	3	600
80	3.4	32	0	4	1380
100	3.4	32	0	5	25536
120	3.4	32	0	6	21192
Experiment 2.		Room Temperature: 23±2 °C			
20	3.4	40	0	1	492
40	3.4	35	0	2	552
60	3.4	35	0	3	1080
80	3.4	34	0	4	2028
100	3.4	33	0	5	27624
120	3.4	32	1	6	26100
Experiment 3.		Room Temperature: 23±2 °C			
20	3.4	40	0	1	168
40	3.4	40	0	2	348
60	3.4	37	0	3	600
80	3.4	36	0	4	1488
100	3.4	35	0	5	25620
120	3.4	32	0	6	22680
Experiment 4.		Room Temperature: 23±2 °C			
20	3.4	39	0	1	564
40	3.4	36	0	2	1104
60	3.4	34	0	3	1800
80	3.4	34	0	4	3408
100	3.4	33	0	5	30468
120	3.4	35	0	6	40368
Experiment 5		Room Temperature: 23±2 °C			
20	3.4	40	0	1	360
40	3.4	40	0	2	660
60	3.4	37	0	3	1116
80	3.4	36	0	4	2400
100	3.4	35	0	5	31860
120	3.4	35	0	6	35436

Untreated filter 1 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.0	38	0	1	720
40	3.0	36	0	2	828
60	3.0	34	0	3	1260
80	3.0	33	0	4	2472
100	3.0	33	0	5	36264
120	3.0	33	0	6	33324
Experiment 2.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	684
40	3.0	37	0	2	1008
60	3.0	34	0	3	1632
80	3.0	33	0	4	3060
100	3.0	33	0	5	42960
120	3.0	32	0	6	37884
Experiment 3.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	660
40	3.0	34	1	2	912
60	3.0	33	0	3	1380
80	3.0	33	0	4	2700
100	3.0	33	0	5	32292
120	3.2	33	0	6	26484
Experiment 4.		Room Temperature: 23±2 °C			
20	3.0	39	0	1	252
40	3.0	35	0	2	396
60	3.0	35	0	3	492
80	3.0	34	0	4	1560
100	3.0	34	0	5	24672
120	3.2	33	0	6	20604
Experiment 5		Room Temperature: 23±2 °C			
20	3.0	40	0	1	408
40	3.0	38	0	2	936
60	3.0	36	1	3	1428
80	3.0	34	0	4	3228
100	3.0	33	0	5	34092
120	3.0	33	0	6	27060

Untreated filter 2 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.0	40	0	1	288
40	3.0	40	0	2	552
60	3.0	38	0	3	612
80	3.0	34	0	4	1368
100	3.0	34	0	5	28644
120	3.0	33	0	6	20724
Experiment 2.		Room Temperature: 23±2 °C			
20	3.0	35	0	1	120
40	3.0	35	0	2	252
60	3.1	35	0	3	648
80	3.2	34	0	4	960
100	3.2	33	0	5	19788
120	3.2	33	0	6	20484
Experiment 3.		Room Temperature: 23±2 °C			
20	3.1	38	1	1	480
40	3.0	33	1	2	816
60	3.0	33	0	3	1260
80	3.0	33	0	4	2412
100	3.0	33	0	5	29232
120	3.0	33	0	6	28572
Experiment 4.		Room Temperature: 23±2 °C			
20	3.0	40	0	1	168
40	3.0	40	0	2	672
60	3.1	38	0	3	1044
80	3.0	36	0	4	2088
100	3.0	33	0	5	30600
120	3.2	33	0	6	35472
Experiment 5		Room Temperature: 23±2 °C			
20	3.1	40	0	1	384
40	3.1	36	0	2	768
60	3.1	34	0	3	1116
80	3.2	33	2	4	2148
100	3.1	34	0	5	29472
120	3.1	34	0	6	22608

Untreated filter 3 (Room temperature & Low RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.0	53.0	0	1	300
40	3.1	53	0	2	456
60	3.1	52	0	3	636
80	3.2	49	0	4	1296
100	3.2	48	0	5	27684
120	3.1	46	0	6	36816
Experiment 2.		Room Temperature: 23±2 °C			
20	3.1	40	0	1	144
40	3.1	39	0	2	372
60	3.1	39	0	3	780
80	3.1	39	0	4	1392
100	3.2	38	0	5	28068
120	3.1	38	0	6	29412
Experiment 3.		Room Temperature: 23±2 °C			
20	3.1	40	0	1	276
40	3.1	40	1	2	552
60	3.1	37	0	3	588
80	3.1	36	0	4	2112
100	3.2	35	0	5	25200
120	3.1	36	0	6	30348
Experiment 4.		Room Temperature: 23±2 °C			
20	3.1	37	0	1	276
40	3.1	37	0	2	324
60	3.2	35	0	3	480
80	3.1	35	0	4	1284
100	3.1	36	0	5	24924
120	3.1	36	0	6	21528
Experiment 5		Room Temperature: 23±2 °C			
20	3.1	40	0	1	456
40	3.1	38	1	2	444
60	3.1	36	0	3	744
80	3.2	35	0	4	2136
100	3.2	36	0	5	30924
120	3.2	36	0	6	25944

Iodine-treated filter 1 (Room temperature & High RH)					
Experiment 1.		Room Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	2.6	100	0	1	300
40	2.7	100	0	2	564
60	2.6	100	0	3	1008
80	2.7	100	0	4	1800
100	2.6	100	0	5	32844
120	2.6	100	0	6	44304
Experiment 2.		Room Temperature: 23±2 °C			
20	2.9	100	0	1	456
40	2.5	99	0	2	684
60	2.4	100	0	3	1176
80	2.7	100	0	4	2076
100	2.8	80	0	5	32712
120	2.7	87	0	6	39900
Iodine-treated filter 2 (Room temperature & High RH)					
Experiment 1.		Room Temperature: 23±2 °C			
20	2.7	95	0	1	516
40	2.8	90	0	2	636
60	2.6	91	0	3	1236
80	2.6	90	0	4	2544
100	2.8	92	0	5	33804
120	2.8	93	0	6	34056
Experiment 2.		Room Temperature: 23±2 °C			
20	2.8	92	1	1	480
40	2.6	86	0	2	804
60	2.6	91	0	3	1128
80	2.8	92	0	4	2220
100	3.0	96	0	5	32712
120	2.8	93	0	6	42312

Iodine-treated filter 1 (High temperature & High RH)					
Experiment 1.		High Temperature : 40±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Penetration (CFU)	Baseline	
				Impactor	CFU
20	3.0	100	0	1	420
40	3.5	100	0	2	888
60	3.4	100	0	3	1224
80	3.6	100	0	4	2544
100	3.4	100	0	5	36264
120	3.6	100	0	6	45660
Experiment 2.		High Temperature : 40±2 °C			
20	3.8	92	0	1	660
40	3.4	86	1	2	732
60	3.0	91	0	3	1152
80	2.1	92	0	4	2892
100	2.6	96	0	5	39264
120	2.7	93	0	6	44952
Iodine-treated filter 2 (High temperature & High RH)					
Experiment 1.		High Temperature : 40±2 °C			
20	2.8	100	0	1	1080
40	2.7	100	0	2	1464
60	2.7	100	0	3	2052
80	2.5	100	0	4	3804
100	2.6	96	0	5	36996
120	2.8	98	0	6	47652
Experiment 2.		High Temperature : 40±2 °C			
20	2.7	98	0	1	684
40	2.7	100	0	2	1188
60	3.0	100	0	3	1728
80	2.7	100	0	4	3708
100	2.8	96	0	5	37140
120	2.8	100	0	6	47652

Appendix D. Pressure Drag Calculation

- Glass fiber filters (Millipore AP 15)
  - Air resistance at 10.5 fpm : 210 mm H_2O [58]

$$S = \frac{\Delta P}{V_f} = \frac{2058Pa}{0.053m/\sec} = 38830Pa/(m/\sec) \approx 4\times10^4\,Pa/(m/\sec)$$

Pressure drop: $\Delta P = 210mmH_2O \times \frac{9.8Pa}{mmH_2O} = 2058Pa$

Filter velocity: $V_f = 10.5\frac{ft}{\min} \times \frac{0.3048m}{ft} \times \frac{\min}{60\sec} = 0.053m/\sec$

- Filters for bacterial spore aerosols experiment
  - Air resistance at 15 Lpm : 3.0 in H_2O

$$S = \frac{\Delta P}{V_f} = \frac{747Pa}{0.142m/\sec} = 5261Pa/(m/\sec) \approx 6\times10^3\,Pa/(m/\sec)$$

Pressure drop: $\Delta P = 3.0inH_2O \times \frac{249Pa}{inH_2O} = 747Pa$

Filter velocity: $V_f = 14.2\frac{cm}{\sec} \times \frac{0.01m}{cm} = 0.142m/\sec$

Appendix E. Raw Data of Virus Experiments

Iodine-treated filter 1 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	38	2605	48350	94.61
60	0.3	39	2190	25950	91.56
90	0.3	39	2000	52550	96.19
120	0.2	38	1215	23950	94.93
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	1215	23950	94.93
60	0.3	38	610	21300	97.14
90	0.2	38	570	21400	97.34
120	0.2	37	995	58650	98.30
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	625	7350	91.50
60	0.2	39	2155	20800	89.64
90	0.2	39	455	10150	95.52
120	0.2	38	1315	31300	95.80
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	280	9900	97.17
60	0.2	38	410	4900	91.63
90	0.2	39	430	4250	89.88
120	0.3	37	200	6650	96.99
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	305	3000	89.83
60	0.2	38	120	1400	91.43
90	0.2	38	100	1650	93.94
120	0.2	37	500	11550	95.67

Iodine-treated filter 2 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	1090	15550	92.99
60	0.2	39	405	5350	92.43
90	0.2	39	795	12650	93.72
120	0.2	39	500	8000	93.75
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	1090	22050	95.06
60	0.2	39	800	21300	96.24
90	0.2	39	465	21100	97.80
120	0.2	40	915	16100	94.32
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	705	9050	92.21
60	0.2	38	1075	47050	97.72
90	0.3	38	1660	28900	94.26
120	0.3	37	1055	15800	93.32
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	125	7600	98.36
60	0.2	39	70	2300	96.96
90	0.2	38	495	8050	93.85
120	0.2	38	720	9450	92.38
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	1100	11800	90.68
60	0.2	39	390	8000	95.13
90	0.2	40	445	5100	91.27
120	0.2	38	320	8400	96.19

Iodine-treated filter 3 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	1490	14450	89.69
60	0.2	39	5770	92900	93.79
90	0.2	39	1950	18850	89.66
120	0.2	39	1815	20400	91.10
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	780	9100	91.43
60	0.2	39	265	6250	95.76
90	0.2	39	335	3750	91.07
120	0.2	40	180	4000	95.50
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	155	2700	94.26
60	0.2	40	355	9250	96.16
90	0.2	39	245	3950	93.80
120	0.2	40	265	3350	92.09
Experiment 4.		Temperature: 23±2 °C			
30	0.2	40	1395	13250	94.26
60	0.2	40	965	20150	96.16
90	0.2	40	395	3650	93.80
120	0.2	40	795	11800	92.09
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	2265	24150	90.62
60	0.2	40	3025	28850	89.51
90	0.2	39	2490	29400	91.53
120	0.2	40	2505	25550	90.20

Untreated filter 1 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	Pressure drop (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	2320	21700	89.31
60	0.2	40	2115	25600	91.74
90	0.2	39	2405	21200	88.66
120	0.2	38	2370	28350	91.64
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	345	7400	95.34
60	0.2	39	550	11650	95.28
90	0.2	38	570	7800	92.69
120	0.2	38	350	7300	95.21
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	3480	47850	92.73
60	0.2	39	2820	29950	90.58
90	0.2	38	1850	26200	92.94
120	0.2	38	2310	28900	92.01
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	540	6000	91.00
60	0.2	38	270	3550	92.39
90	0.2	38	185	3050	93.93
120	0.2	38	315	4950	93.64
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	680	8350	91.86
60	0.2	39	580	7600	92.37
90	0.2	39	455	6200	92.66
120	0.2	38	700	9000	92.22

Untreated filter 2 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	40	430	4000	89.25
60	0.2	39	280	4250	93.41
90	0.2	39	445	4400	89.89
120	0.2	38	275	3200	91.41
Experiment 2.		Temperature: 23±2 °C			
30	0.2	39	1660	10650	84.41
60	0.2	39	515	9400	94.52
90	0.2	38	1110	10200	89.12
120	0.2	38	490	5350	90.84
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	915	8450	89.17
60	0.2	39	595	5600	89.38
90	0.2	38	1365	18500	92.62
120	0.2	38	730	9400	92.23
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	1705	17050	90.00
60	0.2	39	805	8350	90.36
90	0.2	39	865	7800	88.91
120	0.2	38	710	9150	92.24
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	1480	22300	93.36
60	0.2	39	1220	15050	91.89
90	0.2	40	535	5250	89.81
120	0.2	38	415	4800	91.35

Untreated filter 3 (Room temperature & Low RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	39	450	6350	92.91
60	0.2	39	265	8000	96.69
90	0.2	38	795	12950	93.86
120	0.2	38	1210	28950	95.82
Experiment 2.		Temperature: 23±2 °C			
30	0.2	40	1680	15400	89.09
60	0.2	40	2600	20750	87.47
90	0.2	39	770	13450	94.28
120	0.2	38	550	6350	91.34
Experiment 3.		Temperature: 23±2 °C			
30	0.2	40	690	6300	89.05
60	0.2	39	665	8050	91.74
90	0.2	38	935	9100	89.73
120	0.2	38	490	5450	91.01
Experiment 4.		Temperature: 23±2 °C			
30	0.2	39	880	11050	92.04
60	0.2	39	710	8350	91.50
90	0.2	39	675	9200	92.66
120	0.2	38	660	9150	92.79
Experiment 5.		Temperature: 23±2 °C			
30	0.2	40	880	13950	93.69
60	0.2	38	900	10550	91.47
90	0.2	38	585	7700	92.40
120	0.2	38	640	8800	92.73

Iodine-treated filter 1 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.5	56	90	17700	99.49
60	0.3	51	40	10700	99.63
90	0.3	50	20	16900	99.88
120	0.3	53	0	8800	100.00
Experiment 2.		Temperature: 23±2 °C			
30	0.3	60	1	5150	99.98
60	0.2	53	0	5100	100.00
90	0.3	51	3	2650	99.89
120	0.3	56	3	4750	99.94
Experiment 3.		Temperature: 23±2 °C			
30	0.3	57	0	2500	100.00
60	0.2	51	65	6950	99.06
90	0.2	51	55	9350	99.41
120	0.2	51	10	7050	99.86
Experiment 4.		Temperature: 23±2 °C			
30	0.3	49	10	2150	99.53
60	0.3	52	20	2300	99.13
90	0.3	60	20	4200	99.52
120	0.3	57	0	4000	100.00
Experiment 5.		Temperature: 23±2 °C			
30	0.2	39	0	3450	100.00
60	0.2	38	0	1100	100.00
90	0.2	38	0	4150	100.00
120	0.2	37	0	2350	100.00

Iodine-treated filter 2 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	58	0	3000	100.00
60	0.3	54	0	6000	100.00
90	0.2	59	0	1850	100.00
120	0.2	56	0	2000	100.00
Experiment 2.		Temperature: 23±2 °C			
30	0.3	56	0	950	100.00
60	0.2	54	0	3650	100.00
90	0.3	57	10	700	98.57
120	0.3	56	10	300	96.67
Experiment 3.		Temperature: 23±2 °C			
30	0.2	57	0	3100	100.00
60	0.3	59	1	2150	99.95
90	0.3	57	0	1400	100.00
120	0.3	59	0	1450	100.00
Experiment 4.		Temperature: 23±2 °C			
30	0.2	55	0	550	100.00
60	0.2	57	0	700	100.00
90	0.2	56	0	1700	100.00
120	0.2	57	0	2050	100.00
Experiment 5.		Temperature: 23±2 °C			
30	0.3	69	0	1750	100.00
60	0.3	59	0	1750	100.00
90	0.3	60	0	2300	100.00
120	0.3	58	0	600	100.00

Untreated filter 1 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	47	1100	43000	97.44
60	0.3	46	900	20000	95.50
90	0.3	45	1000	19000	94.74
120	0.3	45	1300	21000	93.81
Experiment 2.		Temperature: 23±2 °C			
30	0.3	43	6350	121000	94.75
60	0.2	47	10100	178000	94.32
90	0.3	46	9050	101500	91.08
120	0.3	45	9950	245000	95.94
Experiment 3.		Temperature: 23±2 °C			
30	0.3	46	4150	41000	89.88
60	0.3	45	4300	44000	90.23
90	0.3	46	4150	55000	92.46
120	0.3	46	3150	72000	95.63
Experiment 4.		Temperature: 23±2 °C			
30	0.3	51	1000	14050	92.88
60	0.3	49	685	12400	94.48
90	0.3	47	925	14550	93.64
120	0.3	48	1000	17500	94.29
Experiment 5.		Temperature: 23±2 °C			
30	0.3	50	3050	33500	90.90
60	0.3	46	1800	22500	92.00
90	0.3	45	1600	26500	93.96
120	0.3	44	2900	30000	90.33

Untreated filter 2 (Room temperature & Medium RH)					
Experiment 1.		Temperature: 23±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.2	48	3150	33000	90.46
60	0.2	47	1850	21150	91.25
90	0.2	47	1650	16200	89.82
120	0.2	47	750	16700	95.51
Experiment 2.		Temperature: 23±2 °C			
30	0.2	47	1000	14800	93.24
60	0.2	48	1650	15200	89.15
90	0.2	47	1900	19000	90.00
120	0.2	45	1100	19000	94.21
Experiment 3.		Temperature: 23±2 °C			
30	0.2	49	2650	28500	90.70
60	0.2	47	3600	39000	90.77
90	0.2	47	2650	35500	92.54
120	0.2	45	3350	31500	89.37
Experiment 4.		Temperature: 23±2 °C			
30	0.2	47	1300	16350	92.05
60	0.2	49	1350	16250	91.69
90	0.2	45	1900	13500	85.93
120	0.2	45	1700	24200	92.98
Experiment 5.		Temperature: 23±2 °C			
30	0.2	47	3250	39500	91.77
60	0.2	47	4050	44000	90.80
90	0.2	44	2100	25500	91.77
120	0.2	45	3700	41500	91.08

Iodine-treated filter 1 (High temperature & Low RH)					
Experiment 1.		Temperature: 40±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	39	0	800	100.00
60	0.3	38	0	445	100.00
90	0.3	38	0	26500	100.00
120	0.3	40	0	38000	100.00
Experiment 2.		Temperature: 40±2 °C			
30	0.3	40	0	47500	100.00
60	0.3	39	0	43500	100.00
90	0.3	39	0	40500	100.00
120	0.3	38	0	41000	100.00
Experiment 3.		Temperature: 40±2 °C			
30	0.3	39	0	53500	100.00
60	0.3	38	0	50000	100.00
90	0.3	38	0	57500	100.00
120	0.3	38	0	58500	100.00
Experiment 4.		Temperature: 40±2 °C			
30	0.3	39	0	54500	100.00
60	0.3	39	0	62000	100.00
90	0.3	40	0	28500	100.00
120	0.3	38	0	40500	100.00
Experiment 5.		Temperature: 40±2 °C			
30	0.3	39	0	16000	100.00
60	0.3	40	0	19500	100.00
90	0.3	39	0	18500	100.00
120	0.3	39	0	17500	100.00

Iodine-treated filter 2 (High temperature & Low RH)					
Experiment 1.		Temperature: 40±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	39	1	8300	99.99
60	0.3	38	0	6150	100.00
90	0.3	39	1	9200	99.99
120	0.3	34	0	11300	100.00
Experiment 2.		Temperature: 40±2 °C			
30	0.3	42	1	5150	99.98
60	0.2	41	0	5100	100.00
90	0.3	37	1	2650	99.96
120	0.3	32	3	4750	99.94
Experiment 3.		Temperature: 40±2 °C			
30	0.2	40	0	20000	100.00
60	0.2	32	1	17000	99.99
90	0.2	36	1	14000	99.99
120	0.2	38	1	21500	99.99
Experiment 4.		Temperature: 40±2 °C			
30	0.3	34	0	2800	100.00
60	0.3	35	0	1550	100.00
90	0.3	35	0	5950	100.00
120	0.3	35	0	4000	100.00
Experiment 5.		Temperature: 40±2 °C			
30	0.3	34	4	3500	99.89
60	0.3	36	1	500	99.80
90	0.3	46	0	2500	100.00
120	0.3	35	1	4450	99.98

Untreated filter 1 (High temperature & Low RH)					
Experiment 1.		Temperature: 40±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	33	3550	101000	96.49
60	0.3	35	6900	74500	90.74
90	0.3	40	3950	102500	96.15
120	0.3	39	4850	119000	95.92
Experiment 2.		Temperature: 40±2 °C			
30	0.3	36	2850	87500	96.74
60	0.2	35	3100	137500	97.75
90	0.3	33	6950	50000	86.10
120	0.3	35	8650	90000	90.39
Experiment 3.		Temperature: 40±2 °C			
30	0.3	40	580	14000	95.86
60	0.3	39	1470	18000	91.83
90	0.3	43	1135	14000	91.89
120	0.3	35	825	14500	94.31
Experiment 4.		Temperature: 40±2 °C			
30	0.4	27	2850	155000	98.16
60	0.2	33	5450	90000	93.94
90	0.3	34	4200	134500	96.88
120	0.3	30	5550	35000	84.14
Experiment 5.		Temperature: 40±2 °C			
30	0.2	41	3400	99500	96.58
60	0.2	40	3400	80500	95.78
90	0.2	33	3000	112000	97.32
120	0.2	37	6000	97000	93.80

Untreated filter 2 (High temperature & Low RH)					
Experiment 1.		Temperature: 40±2 °C			
Time (min)	ΔP (in. H_2O)	Relative Humidity (%)	Experiment (PFU/mL)	Control (PFU/mL)	Removal Eff. (%)
30	0.3	38	2335	16500	85.85
60	0.3	36	360	27000	98.67
90	0.3	38	1705	16000	89.34
120	0.3	37	2430	35000	93.06
Experiment 2.		Temperature: 40±2 °C			
30	0.3	42	1785	29500	93.95
60	0.3	42	895	33500	97.33
90	0.3	43	1065	31500	96.62
120	0.3	38	2210	19000	88.37
Experiment 3.		Temperature: 40±2 °C			
30	0.3	38	2050	34500	94.06
60	0.2	32	1750	29000	93.97
90	0.3	35	2150	32500	93.38
120	0.3	42	2400	35000	93.14
Experiment 4.		Temperature: 40±2 °C			
30	0.3	34	1200	14000	91.43
60	0.3	38	950	8500	88.82
90	0.3	40	850	12500	93.20
120	0.3	39	950	15000	93.67
Experiment 5.		Temperature: 40±2 °C			
30	0.3	25	3050	36500	91.64
60	0.3	29	2900	28500	89.82
90	0.3	39	3000	15000	80.00
120	0.3	40	2100	11500	81.74

Appendix F. Filter Quality Calculation

- Glass fiber filters (Millipore AP 15)
  - ΔP: 2.1 kPa
  - $P \approx 7.39\times10^{-5}$

$$q_F = \frac{\ln(1/P)}{\Delta P} = \frac{\ln\left[1/\left(7.39\times10^{-5}\right)\right]}{2.1kPa} = 4.53\ kPa^{-1}$$

- Filters for viral aerosols experiment
  - ΔP: 0.02 kPa
  - $P \approx 0.72$

$$q_F = \frac{\ln(1/P)}{\Delta P} = \frac{\ln(1/0.72)}{0.02kPa} = 16.43\ kPa^{-1}$$